CARDIAC EFFECTS AND RISK FACTORS ASSOCIATED WITH STATUS EPILEPTICUS

by

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STATEMENT OF DISSERTATION APPROVAL

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ABSTRACT

Status epilepticus (SE), which is characterized by any prolonged and sustained seizure without recovery of consciousness, increases mortality risk during SE and in the 30-day period following seizure cessation. Moreover, these seizures produce intense activation of the sympathetic nervous system (SymNS), diffuse myofilament damage, cardiac dysregulation, and autonomic nervous system (ANS) imbalance. It is proposed that these physiological effects of SE contribute to cardiac contractile dysfunctions, arrhythmias, and sudden death subsequent to seizure cessation. Indeed, clinical reports have indicated an increased occurrence of ventricular arrhythmias at the time of death in SE patients, although the mechanism has not been elucidated. Potentially, a sympathetically mediated mechanism may result in a reversible cardiac dysfunction called cardiac stunning, which persists after a cardiac insult, leaving only subtle myofilament damage with normal blood flow. Neurogenically mediated cardiac stunning results from prolonged sympathetic activation and hypersecretion of catecholamines, which over-stimulate adrenoceptors on cardiac myocytes and induces tachycardic ischemia. These changes cause diffuse myocyte damage, increased susceptibility to arrhythmias, and persistent risk of sudden cardiac death

following the insult. We propose that a similar mechanism(s) may occur during SE, whereby prolonged seizure -induced sympathetic activation produces catecholamine mediated tachycardic ischemia, arrhythmias, myocardial stunning and ultimately an increased risk for mortality. Understanding how SE damages the heart and alters normal cardiac function may allow development of treatment paradigms and pharmacotherapeutic strategies to reduce cardiac morbidity and mortality following SE.

TABLE OF CONTENTS

LIST OF FIGURES

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CHAPTER 1

INTRODUCTION

Status Epilepticus and Mortality Risk Factors

SE, which constitutes a medical emergency, is defined as any protracted or recurrent seizure lasting at least 30 min without recovery of consciousness (DeLorenzo, et al. 1996, Hauser 1990, Treiman, et al. 1998). The onset of clinical SE, however, can be evident within 5 min of persistent seizure activity (Sirven and Waterhouse 2003). Several major causes of SE include acute systemic or neurological insults, metabolic disturbances, hyperthermia, hypoxia, stroke, head trauma, drug or alcohol toxicity, nerve gas exposure, and CNS infection (Lowenstein and Alldredge 1998).

This neurological emergency afflicts approximately 50 out of 100,000 people annually (DeLorenzo, et al. 1996, Lowenstein and Alldredge 1998), with >13% experiencing a recurrent episode (Logroscino, et al. 2005, Lowenstein and Alldredge 1998), 40% developing epilepsy (Shorvon 1994), and 20% of patients dying (Towne, et al. 1994). Further, a variety of life-threatening physiological complications can arise during SE, such as metabolic imbalance, pulmonary edema, cardiac dysfunction (Fountain and

Lothman 1995, Lothman 1990, Simon, et al. 1984), increased SymNS activity (Goodman, et al. 1999), and excessive catecholamine secretion (Shimizu, et al. 2008, Simon, et al. 1984, Walton 1993). Despite these life-threatening conditions, SE-related mortality typically does not occur during or immediately following seizure cessation; on the contrary, over 90% of these deaths occur between 1 and 30 days after SE resolution (DeLorenzo, et al. 1992, Fountain 2000, Logroscino, et al. 2005, Towne, et al. 1994).

Unfortunately, the mechanisms that increase mortality risk following SE are unknown, though literature suggests that cardiac risk factors such as altered autonomic tone, myocardial stunning, and cardiac arrhythmias are important contributors (Aminoff and Simon 1980, Engrand and Crespel 2009, Kreisman, et al. 1993, Manno, et al. 2005, Metcalf, et al. 2009b, Painter, et al. 1993, Walton, et al. 1995). Understanding the relationship between various cardiac risks and complications associated with SE may allow development of therapies to prevent postseizure associated morbidity and mortality.

Sympathetic Nervous System Mediated Control of Cardiac Function

Typically, SymNS mediated release of catecholamines will benefit the cardiovascular system and other tissues during strenuous activity by increasing circulation of oxygenated blood and nutrients. However, prolonged sympathetic drive and excessive amounts of catecholamines can be cardio-

toxic, resulting in cardiac over-stimulation and damage (Rona 1985). In sudden cardiac death, hyperactivation of the SymNS that induces cardiac damage has been proposed as a major mechanism underlying an increased susceptibility to arrhythmias and sudden death (Fountain 2000, Miakotnykh and Antiuf'ev 1991, Stollberger and Finsterer 2004b, Szucs, et al. 2006, Walton 1993). Similarly, the intense and protracted activation of the SymNS observed during SE may produce a sudden and potentially prolonged hypersecretion of catecholamines (Anderson 2003, Dorian 2005, Goldberger 1999, Goodman, et al. 1999, Manno, et al. 2005, Metcalf, et al. 2009b, Simon, et al. 1984, Xydas, et al. 2006) that over-stimulate β-1 adrenergic receptors and induce subtle myofilament damage. However, the effects of protracted seizure activity on cardiac damage and contractile performance have not been systematically evaluated.

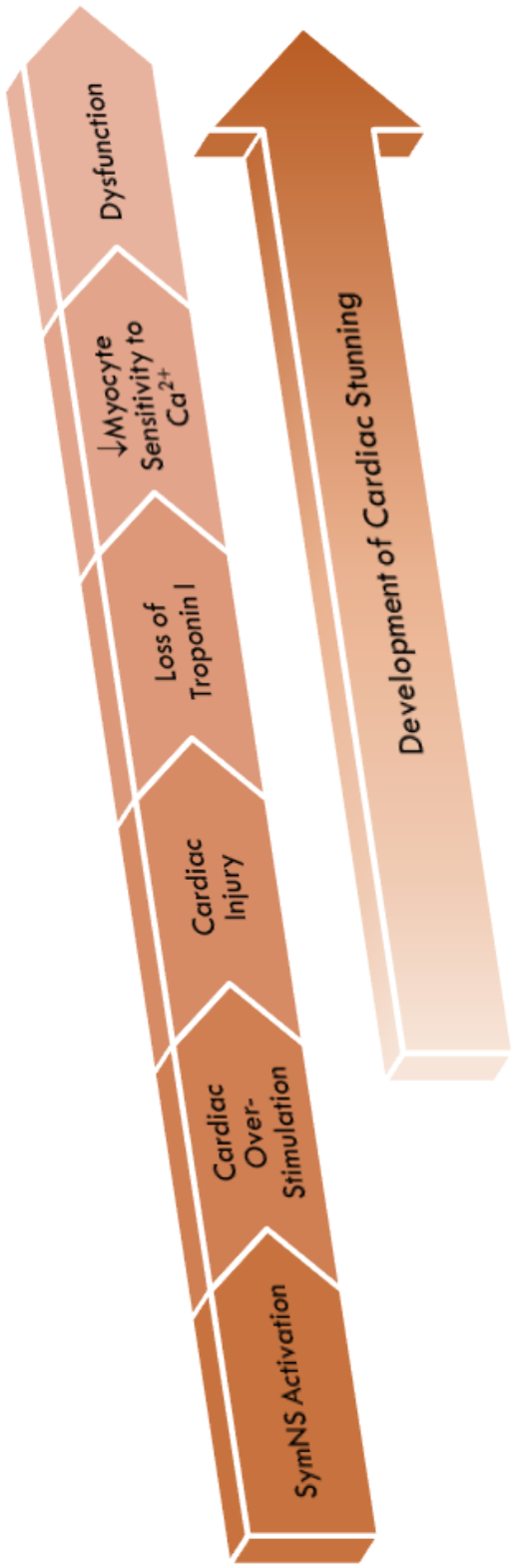
A thorough investigation of cardiac function is needed to formulate appropriate treatment paradigms during SE and post-SE. We propose that SE results in cardiac dysfunction through a neurogenic mechanism, whereby prolonged seizure activity induces SymNS-mediated tachycardia and presents as myocardial stunning. Consequently, in the period following SE, patients who have developed myocardial stunning have an increased risk for arrhythmias and sudden death.

Mechanisms of Neurogenically Mediated Cardiac Stunning

Cardiac stunning (see Fig. 1.1) is a mechanical dysfunction that persists following a reperfusion injury, without irreversible cardiac damage, and despite return of normal cardiac perfusion (Bolli and Marban 1999, Pomblum, et al. 2010). Neurogenically mediated cardiac stunning produces alterations to both systolic and diastolic regional function (Bolli and Marban 1999a, Przyklenk, et al. 1987). Stunning is characterized by a reversible left ventricular dysfunction and increased plasma cardiac troponin I (cTnI) without gross cardiac damage (Bolli 1992, Bolli and Marban 1999, Gao, et al. 1997). Although the long-term prognosis for stunned myocardium is excellent, in the short-term cardiac dysfunction decreases output and alters electrophysiology. These changes increase a patient's risk for lethal arrhythmias and sudden cardiac death during this period (Jain, et al. 2004, Sato, et al. 1999).

Neurogenically-mediated cardiac stunning may result from stress-related activation of the SymNS and catecholamine secretions (Iga, et al. 1995, Lee, et al. 2006). For example, neurally-mediated cardiac stunning has been reported following subarachnoid hemorrhage (Bulsara, et al. 2003, Donaldson and Pritz 2001, Lee, et al. 2006), cerebral infarctions (Wang, et al. 1997), brain tumors (Chuang and Chao 2000), and Guillian-Barre syndrome (Bernstein, et al. 2000). Although the mechanism is not fully understood, it is proposed that high levels of circulating catecholamines and SymNS

Figure 1.1. The mechanism that produces neurogenically mediated cardiac stunning is caused by intense and prolonged activation of the SymNS. This results in protracted periods of catecholamine mediated tachycardic ischemia, hypertension, metabolic demand, and cardiac over-stimulation. Intense adrenergic stimulation causes cardiac injury by means of oxygen radical damage and activation of Ca^{2+} sensitive proteases (calpains), which damage contractile proteins (troponins) involved in excitation-contractile coupling. Damage or loss of functional troponins diminishes myofilament sensitivity to Ca^{2+}, thereby, producing a reversible contractile dysfunction without gross cardiac damage. Recovery from stunning most likely requires repair to excitation-contractile coupling machinery.


SymNS Activation
Cardiac Over-Stimulation
Cardiac Injury
Loss of Troponin I
↓Myocyte Sensitivity to Ca^{2+}
Dysfunction
Development of Cardiac Stunning

overdrive cause excessive stimulation of adrenergic receptors on myocytes. These effects produce prolonged positive inotropic and chronotropic responses that induce tachycardic ischemia (Bolli 1990). During prolonged periods of tachycardia, damaging levels of free radicals may accumulate near myofilaments and high levels of intracellular Ca^{2+} may induce proteolysis of critical contractile proteins such as troponins, through activation of calpains (Bolli 1992, Bolli and Marban 1999, Kloner and Jennings 2001a, Kloner and Jennings 2001b, Kono, et al. 1994). Damage or loss of functional troponins diminishes cardiac function, resulting in decreased myocyte sensitivity to Ca^{2+}, altering both lusitropic and inotropic responses (Bolli 1990, Bolli 1992, Bolli and Marban 1999). Recovery from myocardial stunning, therefore, may depend upon the synthesis of new troponins to restore normal myocardial contractions (Bolli 1992, Jain, et al. 2004). It is proposed that during this recovery period patients have increased risk for sudden cardiac death due to altered cardiac function (Bolli 1992, Bolli and Marban 1999, Kloner, et al. 2001, Pomblum, et al. 2010, Wittstein, et al. 2005). However, it is not clear if recovery of cardiac function results from cardiac remodeling.

The precise mechanism whereby SE produces cardiac stunning has not been fully elucidated. Several studies have demonstrated that SE activates the SymNS, which increases catecholamine release in patients (Shimizu, et al. 2008) and in animals (Sakamoto, et al. 2008, Walton 1993). More recently, neurogenically mediated myocardial stunning has been

reported in patients following protracted seizures (Legriel, et al. 2008, Shimizu, et al. 2008), but has not been demonstrated in animals. Moreover, these reports did not fully test for alterations in cardiac function, recovery, susceptibility to arrhythmias, or mortality risk. Further, these reports did not investigate potential mechanisms of SE-induced cardiac stunning or pursue cardio-protective therapies.

All of the aforementioned pathologies, including SE, have been proposed to produce stunning through a neurogenic mechanism in which sudden and prolonged activation of the SymNS and hypersecretion of catecholamines act to over-stimulate adrenergic receptors on cardiac muscle (Sakamoto, et al. 2008, Shimizu, et al. 2008, Simon 1985). More Specifically, this dissertation addresses the proposal that SE-induced adrenoceptor overstimulation produces reversible cardiac dysfunction that can be characterized by subtle myocyte damage, cardiac conduction abnormalities, increased susceptibility to arrhythmias, and left ventricular dysfunction (Bolli and Marban 1999, Legriel, et al. 2008, Lemke, et al. 2008, Manno, et al. 2005, Metcalf, et al. 2009a, Pomblum, et al. 2010, Walton, et al. 1995).

SE-Induced Effects on Cardiac Regulation and Arrhythmias

Accumulating evidence suggests a strong correlation between an increased occurrence of cardiac abnormities following SE and mortality (Chin, et al. 2004, Logroscino, et al. 2005). In one clinical study, within 24

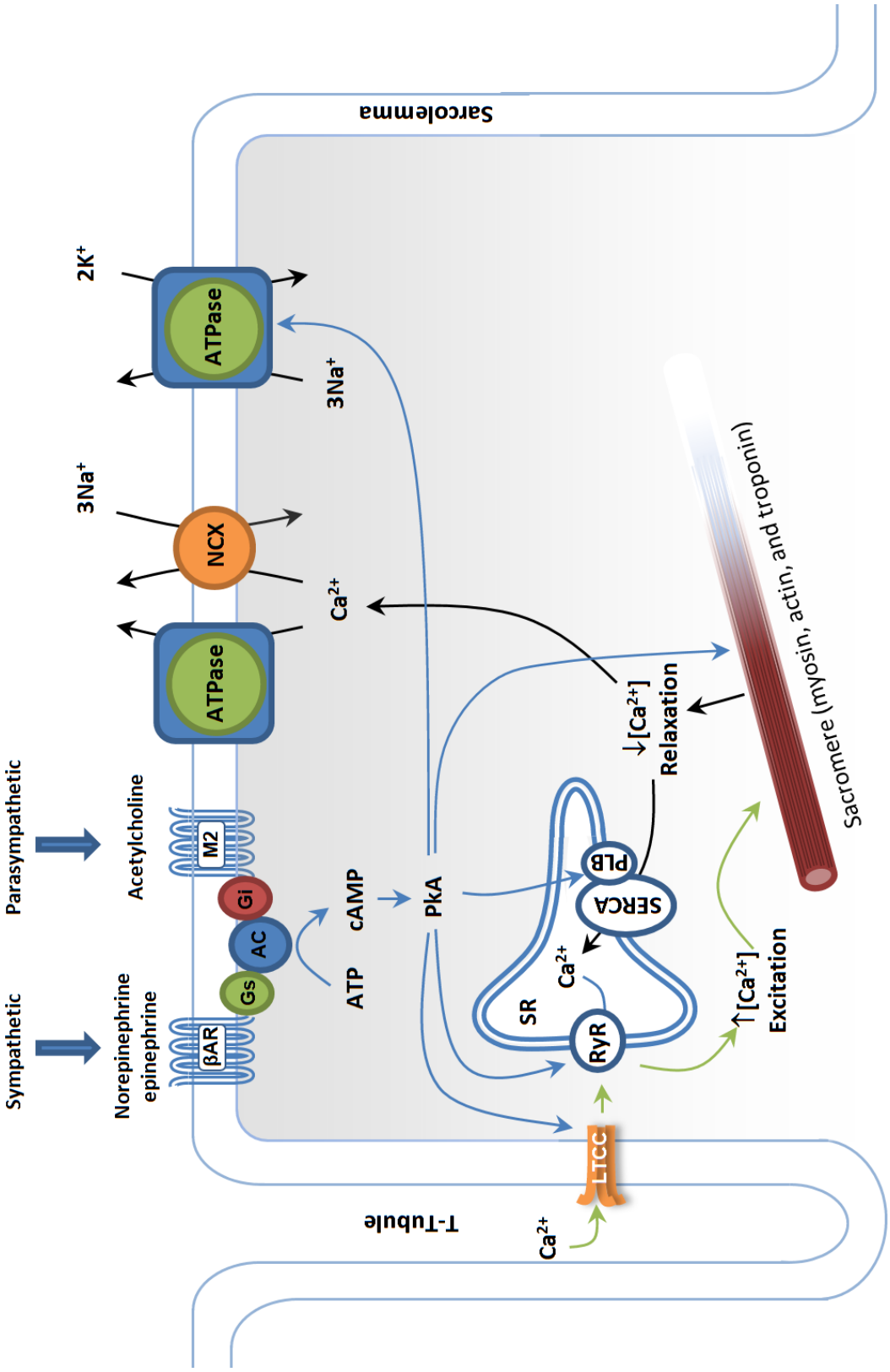
hrs of SE termination over half of patients had specific electrocardiographic (ECG) changes including arrhythmias, conduction abnormalities, and ischemic patterns (Boggs, et al. 1993). Boggs, et al. further identified two groups of SE patients who died of lethal arrhythmias following seizure cessation—those with preexisting coronary artery disease (CAD) and those without CAD (Boggs, et al. 1998). These investigators concluded that in the days and weeks following SE, patients with no prior CAD had acute cardiac decompensation with only subtle myocardial damage (Boggs, et al. 1998), which can increase mortality risk.

Additional clinical reports have observed a sudden and potentially prolonged activation of the SymNS during SE, which is pro-arrhythmic and produces hypersecretion of endogenous catecholamines (Anderson 2003, Dorian 2005, Goldberger 1999, Goodman, et al. 1999, Manno, et al. 2005, Simon, et al. 1984, Xydas, et al. 2006). This over-stimulates ß-adrenergic receptors on cardiac muscle (Sakamoto, et al. 2008, Shimizu, et al. 2008, Simon 1985), elevating both HR and BP (Goodman, et al. 1990, Johnston, et al. 1997, Sakamoto, et al. 2008) for the duration of the seizure. Unfortunately, the mechanisms that produces subtle cardiac damage and increased susceptibility to ventricular arrhythmias following SE are not known. Ventricular arrhythmias are well established as a major cause of sudden cardiac death following neural activation and can result from myofilament damage, diastolic Ca^{2+} dysregulation, and changes in ANS

function (Davis and Natelson 1993, Fredericks, et al. 2007, Hall 1983, Manno, et al. 2005). As discussed above, one potential effect of SE-induced myofilament damage is cardiac stunning (Davis and Natelson 1993, Engrand and Crespel 2009, Goodman, et al. 1990, Lathers and Schraeder 1987, Manno, et al. 2005, Robakis and Hirsch 2006, Shimizu, et al. 2008, Stollberger and Finsterer 2004a, Walton 1993, Young, et al. 1985).

Cardiac stunning is a contractile dysfunction that may result from decreased sensitivity to Ca^{2+} caused by myocyte damage (Gao, et al. 1995). This is because myocytes are entirely dependent on Ca^{2+} to regulate myofilament contraction and relaxation. For cardiac myocytes to function normally, cytosolic Ca^{2+} concentrations need to be rigorously controlled (see Fig. 1.2). During the myocyte action potential, extra cellular Ca^{2+} enters the myocyte through depolarization-activated (voltage-gated) ion channels producing inward Ca^{2+} currents. These inward Ca^{2+} currents trigger ryanodine receptors to release Ca^{2+}, which is bound to calsequestrin, from the sarcoplasmic reticulum (SR)—this is known as Ca^{2+} induced Ca^{2+} release. Increased cytosolic Ca^{2+} levels bind to troponin C in the sarcomere, causing a conformational change that moves tropomyosin out of the way so that the myosin cross bridges can attach to actin and produce muscle contraction—this is known as excitation-contraction coupling. Conversely, relaxation of the sarcomere is triggered by the removal of cytosolic Ca^{2+} (this is the period of diastolic Ca^{2+} decline) through plasma membrane ATPases, Na^{+}/Ca^{2+}

Figure 1.2. Ca^{2+} cycling in myocytes (see text for details). ß-adrenergic receptor, ßAR; muscarinic-2 receptor, M2; adenyl cyclase, AC; Na^{+}/Ca^{2+} exchanger, NCX; sarcoplasmic reticulum, SR; ryanodine receptor, RyR; sarcoplasmic-endoreticulum Ca^{2+} ATPase, SERCA; L-type Ca^{2+} channel, LTCC.


Sarcolemma
T-Tubule
Sacromere (myosin, actin, and troponin)
Parasympathetic
Acetylcholine
M2
Gi
AC
Gs
ATP
cAMP
PkA
Sympathetic
Norepinephrine
epinephrine
βAR
LTCC
SR
RyR
SERCA
PLB
↑[Ca2+]
Excitation
↓[Ca2+]
Relaxation
NCX
ATPase
3Na+
2K+
Ca2+

exchangers, and sequestration back into the SR via sarcoplasmic-endoreticulum Ca^{2+} ATPase (SERCA). Thus, the rate and concentration of Ca^{2+} that enters and exits the cytosol, which is primarily mediated by the SR, directly affects contraction and relaxation of myofilaments. Changes in Ca^{2+} movement, and thereby cardiac function, are regulated by phyosphorylation of various pumps, ions channels, and proteins on both the plasma membrane and the SR. One important regulatory mechanism is SymNS mediated ß-adrenergic stimulation by catecholamines, which is a stimulator G-protein coupled receptor that activates adenylate cyclase, producing cyclic AMP (cAMP) and activation of protein kinase A (PKA). PKA phospharlytes the Na^{+}/Ca^{2+} exchanger, L-type Ca^{2+} channels, phospholamban, and ryanodine receptors resulting in increased sensitivity to depolarization, faster efflux of Ca^{2+} out of the cell, and faster influx of Ca^{2+} into the SR. This results in increase inotropy and chronotropy. ParaSymNS mediated activation of Muscarinic receptors, which is an inhibitory G-protein coupled receptor that deactivates adenylate cylcase and opposes ß-adrenergic stimulation, reduces cAMP and PKA levels causing decreased inotropy and chronotorpy. Clearly, Ca^{2+} is an essential messenger that regulates cardiac electrical activity and is a direct activator of cardiac conduction, contraction and relaxation. Moreover, alterations in Ca^{2+} regulation can alter cardiac function and have been proposed as a major mechanism by which cardiac dysfunctions like cardiac stunning occur (Bolli and Marban 1999).

Studies by Gao, et al. suggest that enhanced susceptibility to Ca^{2+} overload, which is an accumulation of intracellular Ca^{2+} that occurs during protracted tachycardia, supports the functional deterioration of myocardial stunning during intense inotropic stimulation (Gao, et al. 1995) and increases the arrhythmogenic activity of the heart by altering cardiac electrical activity and function (Anderson 2003, Bernstein, et al. 2000, Devinsky 2004, Freeman 2006, Gao, et al. 1995, Lathers and Schraeder 1987, Metcalf, et al. 2009b, Nei, et al. 2000, Opherk, et al. 2002, Ryvlin, et al. 2006, Teplitz, et al. 2005). However, there has not yet been any evaluation of diastolic Ca^{2+} regulation in myocytes during or after SE. By understanding the underling mechanism of SE-induced cardiac dysfunction, such as Ca^{2+} dysregulation and damaged excitation-coupling machinery, cardiac therapies and prognostic indicators could be identified that might lead to better prediction of who might be susceptible to seizure-related cardiac arrhythmias and mortality. Moreover, the advent of a reliable biomarker would be a substantial advance in the prevention of death following SE.

Another mechanism by which increased susceptibility to arrhythmias may occur following SE is altered ANS balance. Sympathetic tone is the primary mechanism regulating left ventricular contraction (Fozzard 1986). Activation of the SymNS elevates endogenous levels of catecholamines, which act to increase blood pressure (BP), heart rate (HR), and myocardial contraction (see Fig. 1.2). Several reports have shown that generalized

seizures produce widespread and undifferentiated activation of the SymNS (Doba, et al. 1975, Goodman, et al. 1999). The acute effects of hyperactivation of the SymNS result in catecholamine levels that are in the arrhythmogenic range and may contribute to sudden death (Simon, et al. 1984). Although the long-term post-SE effects on autonomic control and cardiac function are poorly understood, recent observations from our lab have demonstrated a shift toward sympathovagal tone resulting from paraSymNS withdrawal (Metcalf, et al. 2009b). However, it is not clear if the increase in SymNS tone is a direct affect of damaged ANS brain centers, changes in afferent input, altered SymNS innervations of the heart, or unresponsive ß-adrenergic receptors.

Importantly, an increase in SymNS tone combined with contractile dysfunction greatly increases the risk of lethal cardiac arrhythmias and sudden death (Lhatoo and Sander 2002, Stollberger and Finsterer 2004b, Szucs, et al. 2006, Thom, et al. 2003). Thus, it is proposed that catecholamine overstimulation of cardiac receptors during SE and ANS imbalance post-SE may represent mechanisms that contribute to stunned myocardium and increased susceptibility to lethal ventricular arrhythmias in the period following seizure cessation.

Cardioprotective Pharmacotherapy

In the proposed mechanism of cardiac stunning, an increase in catecholamines, Ca^{2+} dysregulation, and generation of oxygen radicals in myocytes induce damage and proteolysis of troponins. Damage or loss of functional troponins diminishes cardiac function by decreasing myofilament sensitivity to Ca^{2+} and muscle contractility. The literature supports this cascade of events by demonstrating that therapy with a ß-1 adrenergic receptor antagonist (Kyuma, et al. 2002, Vittone, et al. 2006, Yoshioka, et al. 2008) may function to limit tachycardia caused by elevated adrenergic stimulation, thereby effectively decreasing SymNS tone on the heart (Adamson and Gilbert 2006, Dorian 2005). We propose that cardioprotective therapy with an adrenergic blockade will provide protection from tachycardic ischemia and myofilament damage during SE, thereby preventing stunned myocardium, increased susceptibility to lethal ventricular arrhythmias, and decreased SE-associated mortality.

Research Objectives

SE is associated with significant mortality in the days and weeks following the acute event. It has been proposed that SE-induced deaths result from subtle cardiac deficits and increased susceptibly to lethal arrhythmias; however, the mechanism(s) remains unknown. One potential explanation for the development of lethal ventricular arrhythmias

subsequent to an episode of SE is cardiac stunning. Stunning results from altered ANS regulation of the heart, catecholamine-induced cardio-toxicity, over stimulation of myocytes, diastolic Ca^{2+} overload, and loss of functional troponins (Boggs, et al. 1998, Boggs, et al. 1993, Manno, et al. 2005, Walton 1993). Therefore, we propose that the hypersecretion of catecholamines during SE produces reversible decrements in cardiac function in the days and weeks following seizure cessation that is consistent with neurogenically-mediated cardiac stunning, which can be prevented or attenuated with a ß-1 adrenergic antagonist. Further, we hypothesize that myocyte damage caused by tachycardic ischemia during SE increases susceptibility to lethal arrhythmias through enhanced susceptibility to Ca^{2+} overload. The following aims will be addressed in testing these hypotheses:

1) determine if SE produces cardiac damage, increased susceptibility to arrhythmias, and diminishes cardiac hemodynamic and functional parameters that are consistent with neurogenically mediated cardiac stunning;
2) determine if cardiac deficits and increased susceptibility to arrhythmias produced by SE are prevented by ß-1 adrenergic antagonist therapy;
3) determine if SE results in intracellular myocyte Ca^{2+} dysregulation, which predisposes rats to arrhythmias.

The following Chapters of this dissertation provide the methods, results, and conclusions of studies that address these aims. Chapter 2 discusses the mechanism mediating detrimental cardiac effects of SE. Chapter 3 discusses the cardio-protective effects of ß-1 adrenergic antagonist therapy during SE. Chapter 4 addresses MRI scans of LV dysfunction following SE and recovery. Chapter 5 discuss *ex vivo* measurements of Ca^{2+} transients following status epilepticus. Chapter 6 provides the overall finding of this dissertation and future directions.

References

Adamson PB, Gilbert EM. (2006) Reducing the risk of sudden death in heart failure with beta-blockers. *J Card Fail* 12:734-746.

Aminoff MJ, Simon RP. (1980) Status epilepticus. Causes, clinical features and consequences in 98 patients. *Am J Med* 69:657-666.

Anderson KP. (2003) Sympathetic nervous system activity and ventricular tachyarrhythmias: recent advances. *Ann Noninvasive Electrocardiol* 8:75-89.

Bernstein R, Mayer SA, Magnano A. (2000) Neurogenic stunned myocardium in Guillain-Barre syndrome. *Neurology* 54:759-762.

Boggs JG, Marmarou A, Agnew JP, Morton LD, Towne AR, Waterhouse EJ, Pellock JM, DeLorenzo RJ. (1998) Hemodynamic monitoring prior to and at the time of death in status epilepticus. *Epilepsy Res* 31:199-209.

Boggs JG, Painter JA, DeLorenzo RJ. (1993) Analysis of electrocardiographic changes in status epilepticus. *Epilepsy Res* 14:87-94.

Bolli R. (1990) Mechanisms of myocardial "stunning". *Circulation* 82:173-738.

Bolli R. (1992) Myocardial 'stunning' in man. *Circulation* 86:1671-1691.

Bolli R, Marban E. (1999) Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* 79:609-634.

Chin RF, Neville BG, Scott RC. (2004) A systematic review of the epidemiology of status epilepticus. *Eur J Neurol* 11:800-810.

Davis AM, Natelson BH. (1993) Brain-heart interactions. The neurocardiology of arrhythmia and sudden cardiac death. *Tex Heart Inst J* 20:158-169.

DeLorenzo RJ, Hauser WA, Towne AR, Boggs JG, Pellock JM, Penberthy L, Garnett L, Fortner CA, Ko D. (1996) A prospective, population-based epidemiologic study of status epilepticus in Richmond, Virginia. *Neurology* 46:1029-1035.

DeLorenzo RJ, Towne AR, Pellock JM, Ko D. (1992) Status epilepticus in children, adults, and the elderly. *Epilepsia* 33 Suppl 4:S15-25.

Devinsky O. (2004) Effects of Seizures on Autonomic and Cardiovascular Function. *Epilepsy Curr* 4:43-46.

Doba N, Beresford HR, Reis DJ. (1975) Changes in regional blood flow and cardiodynamics associated with electrically and chemically induced epilepsy in cat. *Brain Res* 90:115-132.

Dorian P. (2005) Antiarrhythmic action of beta-blockers: potential mechanisms. *J Cardiovasc Pharmacol Ther* 10 Suppl 1:S15-22.

Engrand N, Crespel A. (2009) [Pathophysiologic basis of status epilepticus]. *Rev Neurol (Paris)* 165:315-319.

Fountain NB. (2000) Status epilepticus: risk factors and complications. *Epilepsia* 41 Suppl 2:S23-30.

Fountain NB, Lothman EW. (1995) Pathophysiology of status epilepticus. *J Clin Neurophysiol* 12:326-342.

Fozzard HA. (1986) *The Heart and cardiovascular system : scientific foundations*. Raven Press, New York.

Fredericks S, Degens H, McKoy G, Bainbridge K, Collinson PO, Coulton G, Elmahdi H, Holt DW. (2007) Effect of denervation on the content of cardiac troponin-T and cardiac troponin-I in rat skeletal muscle. *Clin Biochem* 40:423-426.

Freeman R. (2006) Assessment of cardiovascular autonomic function. *Clin Neurophysiol* 117:716-730.

Gao WD, Atar D, Backx PH, Marban E. (1995) Relationship between intracellular calcium and contractile force in stunned myocardium. Direct evidence for decreased myofilament Ca2+ responsiveness and altered diastolic function in intact ventricular muscle. *Circ Res* 76:1036-1048.

Gao WD, Atar D, Liu Y, Perez NG, Murphy AM, Marban E. (1997) Role of troponin proteolysis in the pathogenesis of stunned myocardium. *Circ. Res.* 80:393-399.

Goldberger JJ. (1999) Sympathovagal balance: how should we measure it? *Am J Physiol* 276:H1273-1280.

Goodman JH, Homan RW, Crawford IL. (1990) Kindled seizures elevate blood pressure and induce cardiac arrhythmias. *Epilepsia* 31:489-495.

Goodman JH, Homan RW, Crawford IL. (1999) Kindled seizures activate both branches of the autonomic nervous system. *Epilepsy Res* 34:169-176.

Hall S. (1983) Status epilepticus. *Am Fam Physician* 28:117-121.

Hauser WA. (1990) Status epilepticus: epidemiologic considerations. *Neurology* 40:9-13.

Jain R, Deveikis J, Thompson BG. (2004) Management of patients with stunned myocardium associated with subarachnoid hemorrhage. *AJNR Am J Neuroradiol* 25:126-129.

Johnston SC, Siedenberg R, Min JK, Jerome EH, Laxer KD. (1997) Central apnea and acute cardiac ischemia in a sheep model of epileptic sudden death. *Ann Neurol* 42:588-594.

Kloner RA, Arimie RB, Kay GL, Cannom D, Matthews R, Bhandari A, Shook T, Pollick C, Burstein S. (2001) Evidence for stunned myocardium in humans: a 2001 update. *Coron Artery Dis* 12:349-356.

Kloner RA, Jennings RB. (2001a) Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: part 1. *Circulation* 104:2981-2989.

Kloner RA, Jennings RB. (2001b) Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: part 2. *Circulation* 104:3158-3167.

Kono T, Morita H, Kuroiwa T, Onaka H, Takatsuka H, Fujiwara A. (1994) Left ventricular wall motion abnormalities in patients with subarachnoid hemorrhage: neurogenic stunned myocardium. *J Am Coll Cardiol* 24:636-640.

Kreisman NR, Gauthier-Lewis ML, Conklin SG, Voss NF, Barbee RW. (1993) Cardiac output and regional hemodynamics during recurrent seizures in rats. *Brain Res* 626:295-302.

Kyuma M, Tsuchihashi K, Shinshi Y, Hase M, Nakata T, Ooiwa H, Abiru M, Hikita N, Adachi T, Shoji T, Fujise Y, Shimamoto K. (2002) Effect of intravenous propranolol on left ventricular apical ballooning without coronary artery stenosis (ampulla cardiomyopathy): three cases. *Circ J* 66:1181-1184.

Lathers CM, Schraeder PL. (1987) Review of autonomic dysfunction, cardiac arrhythmias, and epileptogenic activity. *J Clin Pharmacol* 27:346-356.

Legriel S, Bruneel F, Dalle L, Appere-de-Vecchi C, Georges JL, Abbosh N, Henry-Lagarrigue M, Revault D'Allonnes L, Ben Mokhtar H, Audibert J, Guezennec P, Troche G, Bedos JP. (2008) Recurrent takotsubo cardiomyopathy triggered by convulsive status epilepticus. *Neurocrit Care* 9:118-121.

Lemke DM, Hussain SI, Wolfe TJ, Torbey MA, Lynch JR, Carlin A, Fitzsimmons B-FM, Zaidat OO. (2008) Tako-tsubo cardiomyopathy associated with seizures. *Neurocrit. Care*.

Lhatoo SD, Sander JW. (2002) Sudden unexpected death in epilepsy. *Hong Kong Med J* 8:354-358.

Logroscino G, Hesdorffer DC, Cascino G, Hauser WA, Coeytaux A, Galobardes B, Morabia A, Jallon P. (2005) Mortality after a first episode of status epilepticus in the United States and Europe. *Epilepsia* 46 Suppl 11:46-48.

Lothman E. (1990) The biochemical basis and pathophysiology of status epilepticus. *Neurology* 40:13-23.

Lowenstein DH, Alldredge BK. (1998) Status epilepticus. *N Engl J Med* 338:970-976.

Manno EM, Pfeifer EA, Cascino GD, Noe KH, Wijdicks EF. (2005) Cardiac pathology in status epilepticus. *Ann Neurol* 58:954-957.

Metcalf CS, Poelzing S, Little JG, Bealer SL. (2009a) Status epilepticus induces cardiac myofilament damage and increased susceptibility to arrhythmias in rats. *Am J Physiol Heart Circ Physiol* 297:H2120-2127.

Metcalf CS, Radwanski PB, Bealer SL. (2009b) Status epilepticus produces chronic alterations in cardiac sympathovagal balance. *Epilepsia* 50:747-754.

Miakotnykh VS, Antiuf'ev VF. (1991) [Status of the heart conduction system in patients with epileptic seizures]. *Zh Nevropatol Psikhiatr Im S S Korsakova* 91:50-55.

Nei M, Ho RT, Sperling MR. (2000) EKG abnormalities during partial seizures in refractory epilepsy. *Epilepsia* 41:542-548.

Opherk C, Coromilas J, Hirsch LJ. (2002) Heart rate and EKG changes in 102 seizures: analysis of influencing factors. *Epilepsy Res* 52:117-127.

Painter JA, Shiel FO, DeLorenzo RJ. (1993) Cardiac pathology findings in status epilepticus. *Epilepsia* 34 Suppl 6:30.

Pomblum VJ, Korbmacher B, Cleveland S, Sunderdiek U, Klocke RC, Schipke JD. (2010) Cardiac stunning in the clinic: the full picture. *Interact CardioVasc Thorac Surg* 10:86-91.

Robakis TK, Hirsch LJ. (2006) Literature review, case report, and expert discussion of prolonged refractory status epilepticus. *Neurocrit Care* 4:35-46.

Rona G. (1985) Catecholamine cardiotoxicity. *J Mol Cell Cardiol* 17:291-306.

Ryvlin P, Montavont A, Kahane P. (2006) Sudden unexpected death in epilepsy: from mechanisms to prevention. *Curr Opin Neurol* 19:194-199.

Sakamoto K, Saito T, Orman R, Koizumi K, Lazar J, Salciccioli L, Stewart M. (2008) Autonomic consequences of kainic acid-induced limbic cortical seizures in rats: Peripheral autonomic nerve activity, acute cardiovascular changes, and death. *Epilepsia*:1-15.

Sato K, Masuda T, Izumi T. (1999) Subarachnoid hemorrhage and myocardial damage clinical and experimental studies. *Jpn Heart J* 40:683-701.

Shimizu M, Kagawa A, Takano T, Masai H, Miwa Y. (2008) Neurogenic stunned myocardium associated with status epilepticus and postictal catecholamine surge. *Intern. Med.* 47:269-273.

Shorvon S. (1994) *Status epilepticus: its clinical features and treatment in children and adults.* Cambridge Universty Press, Cambridge, England.

Simon RP. (1985) Physiologic consequences of status epilepticus. *Epilepsia* 26 Suppl 1:S58-66.

Simon RP, Aminoff MJ, Benowitz NL. (1984) Changes in plasma catecholamines after tonic-clonic seizures. *Neurology* 34:255-257.

Sirven JI, Waterhouse E. (2003) Management of status epilepticus. *Am Fam Physician* 68:469-476.

Stollberger C, Finsterer J. (2004a) Cardiac troponin levels following monitored epileptic seizures. *Neurology* 62:1453.

Stollberger C, Finsterer J. (2004b) Cardiorespiratory findings in sudden unexplained/unexpected death in epilepsy (SUDEP). *Epilepsy Res* 59:51-60.

Szucs A, Lalit N, Rasonyi G, Barcs G, Bone B, Halasz P, Janszky J. (2006) [Sudden death and mortality in epilepsy]. *Ideggyogy Sz* 59:321-328.

Teplitz L, Igic R, Berbaum ML, Schwertz DW. (2005) Sex differences in susceptibility to epinephrine-induced arrhythmias. *J Cardiovasc Pharmacol* 46:548-555.

Thom M, Seetah S, Sisodiya S, Koepp M, Scaravilli F. (2003) Sudden and unexpected death in epilepsy (SUDEP): evidence of acute neuronal injury using HSP-70 and c-Jun immunohistochemistry. *Neuropathol Appl Neurobiol* 29:132-143.

Towne AR, Pellock JM, Ko D, DeLorenzo RJ. (1994) Determinants of mortality in status epilepticus. *Epilepsia* 35:27-34.

Treiman DM, Meyers PD, Walton NY, Collins JF, Colling C, Rowan AJ, Handforth A, Faught E, Calabrese VP, Uthman BM, Ramsay RE, Mamdani MB. (1998) A comparison of four treatments for generalized convulsive status epilepticus. Veterans Affairs Status Epilepticus Cooperative Study Group. *N Engl J Med* 339:792-798.

Vittone L, Said M, Mattiazzi A. (2006) beta 2-Adrenergic stimulation is involved in the contractile dysfunction of the stunned heart. *Naunyn Schmiedebergs Arch Pharmacol* 373:60-70.

Walton NY. (1993) Systemic effects of generalized convulsive status epilepticus. *Epilepsia* 34 Suppl 1:S54-58.

Walton NY, Rubinstein BK, Treiman DM. (1995) Cardiac hypertrophy secondary to status epilepticus in the rat. *Epilepsy Res* 20:121-124.

Wittstein IS, Thiemann DR, Lima JA, Baughman KL, Schulman SP, Gerstenblith G, Wu KC, Rade JJ, Bivalacqua TJ, Champion HC. (2005) Neurohumoral features of myocardial stunning due to sudden emotional stress. *N Engl J Med* 352:539-548.

Xydas S, Kherani AR, Chang JS, Klotz S, Hay I, Mutrie CJ, Moss GW, Gu A, Schulman AR, Gao D, Hu D, Wu EX, Wei C, Oz MC, Wang J. (2006) beta(2)-Adrenergic stimulation attenuates left ventricular remodeling, decreases apoptosis, and improves calcium homeostasis in a rodent model of ischemic cardiomyopathy. *J Pharmacol Exp Ther* 317:553-561.

Yoshioka T, Hashimoto A, Tsuchihashi K, Nagao K, Kyuma M, Ooiwa H, Nozawa A, Shimoshige S, Eguchi M, Wakabayashi T, Yuda S, Hase M,

Nakata T, Shimamoto K. (2008) Clinical implications of midventricular obstruction and intravenous propranolol use in transient left ventricular apical ballooning (Tako-tsubo cardiomyopathy). *Am Heart J* 155:526 e521-527.

Young RS, Fripp RR, Yagel SK, Werner JC, McGrath G, Schuler HG. (1985) Cardiac dysfunction during status epilepticus in the neonatal pig. *Ann Neurol* 18:291-297.

CHAPTER 2

MECHANISMS MEDIATING DETRIMENTAL CARDIAC EFFECTS OF STATUS EPILEPTICUS

Introduction

Status epilepticus (SE) is a seizure or series of seizures that lasts longer than 30 min and frequently leads to sudden death in the period subsequent to seizure cessation. Even though the underlying mechanism is unknown, lethal cardiac arrhythmias have been implicated at the time of death. We propose as an overriding hypothesis that SE results in cardiac dysfunction through a neurogenically-mediated mechanism, wherein prolonged seizure activity leads to overstimulation of the heart. Such cardiac overstimulation induces a cardiomyopathy that increases the risk for lethal arrhythmias.

Previous work in our lab has already demonstrated that SE results in cardiac damage via SymNS-mediated tachycardia within 90 min, and that SE increases susceptibility to lethal ventricular arrhythmias for at least 14 days following seizure cessation (Metcalf, et al. 2009). These experiments demonstrate that ß-blocker therapy could prevent cardiac damage from

occurring during SE. Further, these results suggest that seizure-induced cardiac injury is a mechanism for mortality during the period following SE. To characterize this potential mechanism, we monitored electrocardiogram (ECG) recordings for altered electrophysiology 10-14 days following SE cessation. Additionally, we evaluated the protective effects of atenolol (AT) administration during SE on ECG alterations and on the susceptibility to lethal arrhythmias at 10-14 days following SE. Cardiac protection with atenolol demonstrated that the mechanism of SE-induced cardiac effects is through SymNS mediated ß-1 adrenergic receptor stimulation.

Methods

Animals

Male Sprague-Dawley rats (225-250g) were obtained from a commercial supplier (Charles River, Wilmington, MA) and maintained at 22°C on a 12hr:12hr, light:dark schedule. Animals were housed two to three per cage in Plexiglass cages before treatment and individually following SE. Animals were allowed *ad libitum* access to standard laboratory rat chow and water. The Institutional Animal Care and Use Committee at the University of Utah approved all experimental procedures.

Induction of SE

SE was induced by sequential administration with lithium and pilocarpine and has been thoroughly described elsewhere (Glien, et al. 2001, Kulkarni and George 1995). Briefly, an injection of lithium (127 mg/kg ip; Sigma, St. Louis, MO) was given 18-24 hrs prior to pretreatment with methyl-scopolamine (2 mg/kg ip, Sigma) for 30 min, followed by administration of pilocarpine (30 mg/kg ip; Sigma) to induce SE. Control (Cont) animals were administered 0.9% saline vehicle in place of pilocarpine. A modified Racine scale (Racine 1972) was used to evaluate seizure activity. Onset of SE was determined by the first grade III or greater seizure followed by more intense and persistent seizures. After 90 min of sustained SE, valproic acid (VPA 400 mg/kg ip; Sigma) was administered to terminate motor seizure activity. Cont animals received injections of VPA at similar time points.

Animal's body weight, eating, and drinking were monitored throughout the experiments for up to 2 weeks. Any hypodypsia and hypophasia were treated with administration of lactated ringer's solution (3mL ip) and softened breakfast cereal (Froot Loops®) in addition to normal laboratory diet.

Catheterization

Twenty-four hours prior to induction of SE or Cont procedures, vascular catheterization was performed. Catheters were used to monitor blood pressure and heart rate during seizure activity; to administer atenolol (a ß-1 adrenergic antagonist) or vehicle (saline); and to obtain blood samples for quantification of plasma cardiac troponin I (cTnI). Catheters consisted of a 40-mm length of PE-10 cemented in PE-50 and filled with heparin/saline (50 U/mL). To implant catheters, animals were anesthetized (Avertin, 300 mg/kg; ip) and shaved on the inside of the right hind limb and the back of the neck. Using blunt dissection, the femoral artery and femoral vein were exposed; next, catheters were inserted to approximately the level of the renal arteries and secured with surgical silk at the point of insertion. All catheters were led subcutaneously to exit between the scapulae. Animals that underwent cardiac testing 10-14 days following SE were implanted with only femoral venous catheters for ß-blocker therapy or vehicle administration during seizures.

Blood Pressure and Heart Rate

Pulsatile BP was measured by a pressure transducer connected directly to the femoral artery catheter and was recorded by a Powerlab Data Acquisition System (ADInstruments) and Macintosh computer. The

computer software (Lab Chart 7; ADInstruments) calculated mean HR and BP from these pulse pressure recordings collected continuously during SE.

ß-Blocker Therapy

ß1-adrenergic antagonist, atenolol (1 mg/kg, iv), or vehicle (saline) was administered to SE or Cont animals immediately prior to the initiation of SE, then subsequently at 30-45 min intervals to maintain heart rate at preseizure levels. Additional atenolol was administered if HR reached >10 bpm from baseline. Once SE was terminated with VPA, no additional atenolol was administered.

Measuring Cardiac Troponin I

Cardiac damage was determined by measuring plasma concentrations of cardiac troponin I (cTnI), which is a well-established blood-borne indicator of cardiac myocyte damage (Antman 2002, O'Brien, et al. 2006, Sarko and Pollack 2002). Plasma samples (100 uL) were collected at 90 min following the initiation of seizure activity. Samples were stored at -80^{o}C until they were used for assays. Plasma cTnI concentrations were measured using a commercially available ELISA kit manufactured for rats (Life Diagnostics, Inc. West Chester, PA).

Evaluation of Cardiac Electrophysiology and Susceptibility to Arrhythmias

Two weeks following SE, both Cont and SE animals were evaluated for changes in cardiac electrophysiology and susceptibility to experimentally induced ventricular arrhythmias by recording ECG activity at baseline and during an infusion of an arrhythmogenic agent. To prepare for cardiac testing, animals were anesthetized (α-chloralose; 40mg/kg, iv bolus; 30mg/kg/hr maintenance iv) and implanted with femoral vein catheters. While animals were still under anesthesia, ECG electrodes were implanted by making two incisions (≈ 10 mm) in the skin of the upper right and lower left quadrants of the chest. The exposed tips (≈ 5 mm) of two insulated silver wires were sewn with surgical silk thread into the thoracic muscles. The wires were placed subcutaneously around the body and externalized between the scapulae. The exposed ends of the wires were soldered to a connector.

On the day following surgery, animals were anesthetized with Urethane (1.2 g/kg, ip). The ECG leads were connected to an amplifier and Powerlab 2/20 recording unit (ADInstruments, Colorado Springs, CO). After a 60 min equilibration period, a 15 min baseline ECG recording was collected (see Fig. 2.1). From these recordings, QT and RR interval measurements were obtained. The QT interval is the total time of ventricular depolarization and repolarization, starting at the beginning of the Q wave and terminating

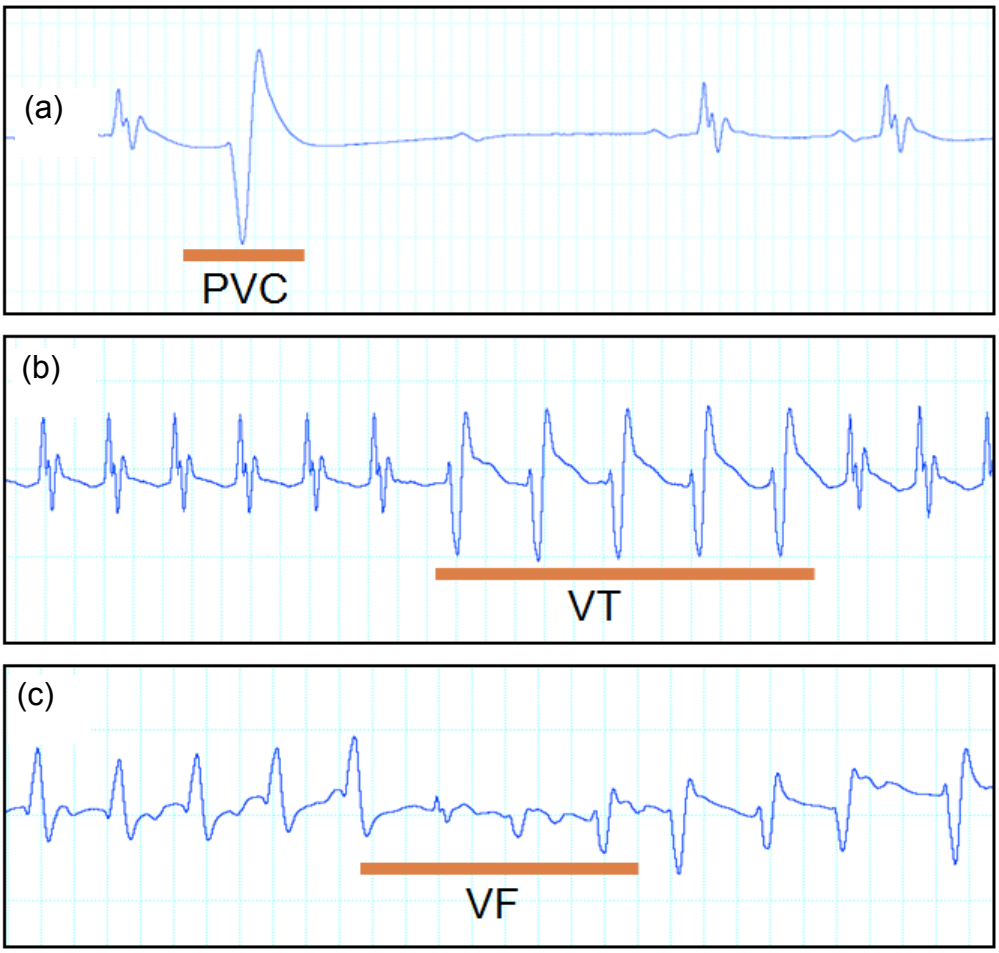


Figure 2.1. Representative ECGs of ventricular arrhythmias induced by aconitine are shown, as follows: (Panel A) premature ventricular contractions (PVC; non-recurring QRS with no P wave); (Panel B) ventricular tachycardia (VT; a minimum sequence of P, QRS, PVC, PVC, PVC, PVC); and (Panel C) ventricular fibrillation (VF; no discernable QRS complex).

when the T-wave returns to the isoelectric value. The RR interval is the time between two successive heart beats. These variables were averaged across 10-20 consecutive heart beats. Bazzett's formula was used to correct the QT interval (QTc) for heart rate, $QTc=QT/(RR)^{1/2}$. QTc dispersion (Qtcd) was calculated by subtracting the minimum QTc from the maximum QTc for each animal. These measurements are well-documented indicators of increased risk for sudden cardiac death (Chugh, et al. 2009, Darbar, et al. 1996, de Bruyne, et al. 1998) and have established procedures for their evaluation in rodents (Chen, et al. 2009, da Silva Costa, et al. 2008, Volk, et al. 2001).

Following baseline cardiac measurements, ECGs were continually recorded to evaluate susceptibility to experimentally-induced arrhythmias using the arrhythmogenic agent aconitine—an irreversible sodium channel agonist. Ventricular arrhythmias (see Fig. 2.1) were evoked by a 7 min infusion aconitine (5 µg/kg/min, iv) using a programmable syringe pump; this method has been shown to produce experimental ventricular arrhythmias in anesthetized rats (Grippo, et al. 2004, Shu, et al. 2004). Susceptibility to arrhythmias was determined by comparing the latency from the initiation of aconitine infusion to onset of: 1) premature ventricular contractions (PVC; non-recurring QRS with no P wave); 2) ventricular tachycardia (VT; a minimum sequence of P, QRS, PVC, PVC, PVC, PVC); and 3) ventricular fibrillation (VF; no discernable QRS complex). These arrhythmias are typically observed when using aconitine (Grippo, et al. 2004, Klekot 2006,

Shu, et al. 2004, Takahara, et al. 1999). Once VF was reached, rats were immediately sacrificed with an high dose of Avertin (1000 mg/kg, iv).

Evaluation of Cerebral Damage Induced by SE

Fluoro-Jade B immunohistochemistry was used to evaluated damage in the dentate gyrus of the hippocampus and used as an additional indicator of seizure intensity in SE animals treated with either atenolol or vehicle (saline). Twenty-four hours following SE termination, animals were anesthetized (Avertin, 300mg/kg; ip) and transcardially perfused with 300 mL of 0.1M neutral phosphate buffered 10% formalin. After this, the brains were rapidly excised and post-fixed overnight in 10% formalin + 20% sucrose. Using a cryostat, 6-8 coronal tissue sections (40 um) were collected from the rostral, mid, and caudal regions of the hippocampus, mounted to charged slides, and allowed to air dry for 30 min. These sections were stained with Fluoro-Jade B following the manufacturer's directions (Chemicon International, Billerica, MA). A confocal microscope equipped with a fluorescein isothiocyanate (FITC) filter (510-560nm band pass) was used to visualize the treated section. Neurodegeneration was quantified in each animal by averaging the number of Fluoro-Jade B positive neurons in all hippocampal sections.

Results

HR and BP

The maximum mean BP and maximum HR during 90 min of seizure activity in SE and Cont animals can be seen in Fig. 2.2. SE animals treated with vehicle (saline) demonstrated a significant increase in both HR and BP in response to prolonged seizure activity. Atenolol administration during SE prevented any increase in HR when compared to Cont, Cont + AT, and SE + AT treated animals, but it did not lower the BP response. No observable difference in seizure intensity observed by atenolol therapy during SE and seizure termination with VPA was similar among all groups.

SE-Induced Cardiac Damage

Fig. 2.3 demonstrates an increase in plasma cTnI within 60 min of SE initiation and indicates cardiac myocyte damage. In contrast, atenolol administration during SE eliminated seizure-related increases in plasma cTnI, which were comparable to Cont, and Cont+AT treated animals. These data demonstrate that SE induces cardiac damage during seizure activity, which can be prevented by β-1 adrenergic receptor blockade.

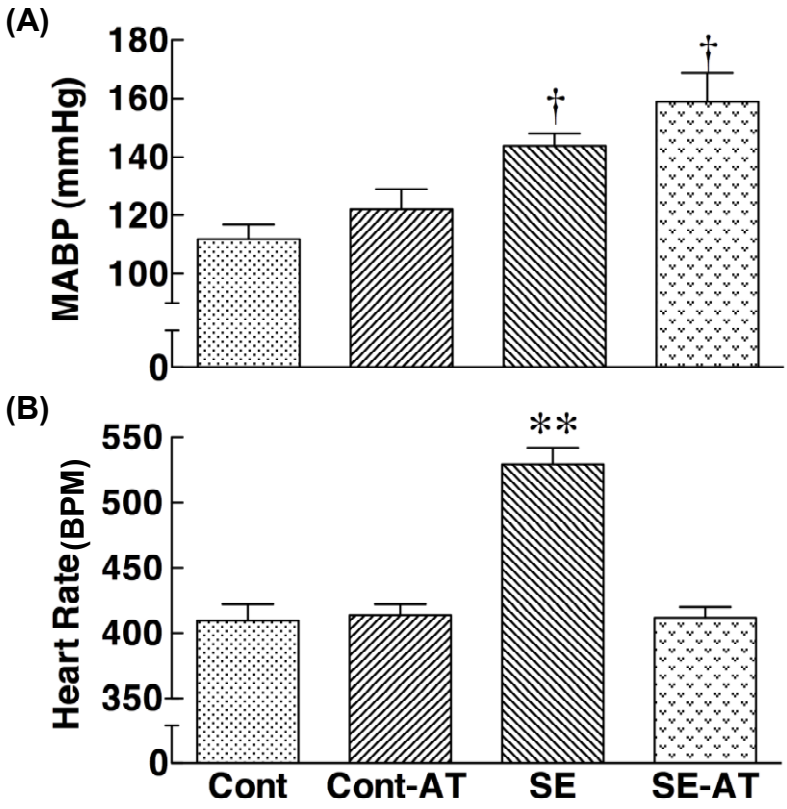


Figure 2.2. Maximum mean arterial blood pressure (Panel A; MABP) and heart rate (Panel B; BPM) during vehicle-treated status epilepticus (SE; n=10) in rats, and at similar time points in Cont (n=7), and in atenolol treated Cont (Cont+AT; n=4) and atenolol treated SE (SE+AT; n=5) animals. †$p<0.01$ compared to Cont and Cont+AT; **$p<0.01$ compared to all other groups.

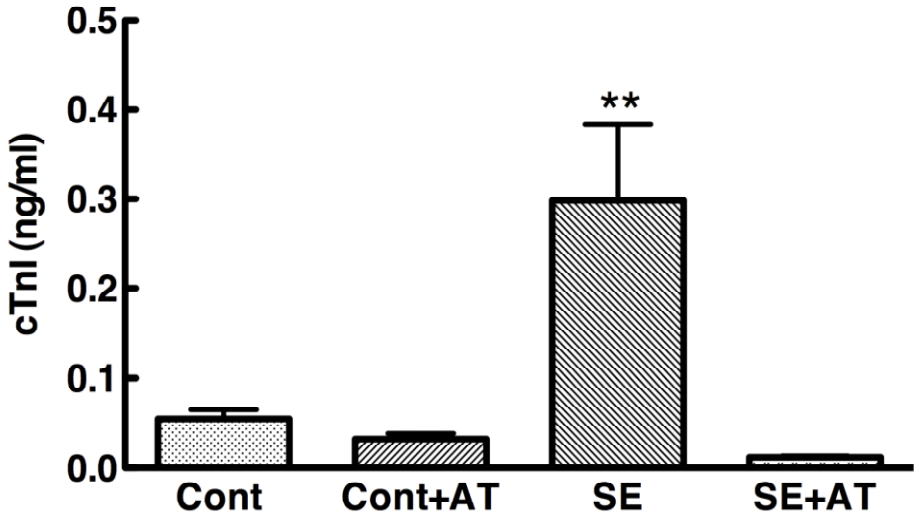


Figure 2.3. Cardiac troponin I (cTnI) plasma concentrations obtained 60 min following the onset of seizure activity in vehicle-treated status epilepticus (SE; n=6) and at similar time points in vehicle-treated control (Cont; n=4) rats, and in atenolol treated Cont (Cont+AT; n=4) and atenolol treated SE (SE+AT; n=5) animals.

Cardiac Electrophysiology—QTc Duration and QTc Dispersion

QT interval prolongation and dispersion are clinically accepted as prognostic indicators of increased risk of arrhythmogenic electrical activity and sudden death. QT interval has been corrected for HR using Bazett's formula. Fig. 2.4 graphically represents the measurements of QTc duration and QTc dispersion, recorded 12-14 days following SE or at equivalent time points in Cont (n=7), Cont+AT (n=7), SE (n=8), and SE+AT (n=7) animals. SE animals demonstrated a significant QTc prolongation and increased QTc dispersion for up to 12-14 days when compared to Cont, Cont + AT, and SE + AT groups. Atenolol administration during SE, however, prevented QTc prolongation and increased QTc dispersion following seizure cessation when compared to vehicle-treated SE animals. These data demonstrate that atenolol is cardioprotective against SE -induced cardiac effects produced through a ß-1 receptor mediated mechanism.

Susceptibility to Arrhythmias

Fig. 2.5 illustrates the mean latencies to aconitine induced ventricular arrhythmias 12-14 days following SE cessation. We previously demonstrated that aconitine infusion in Cont and SE rats resulted in a decreased latency to the 1st PVC , VT and VF, which indicates an increased susceptibility to lethal ventricular arrhythmias (Metcalf, et al. 2009). Atenolol administration during SE, however, prevented the decrease in latency to ventricular

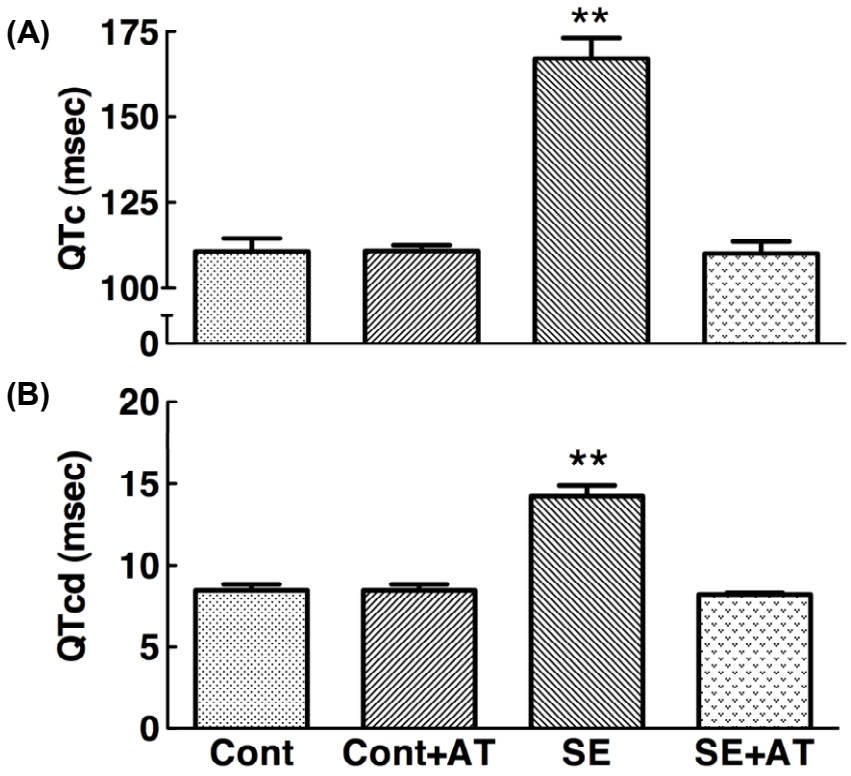
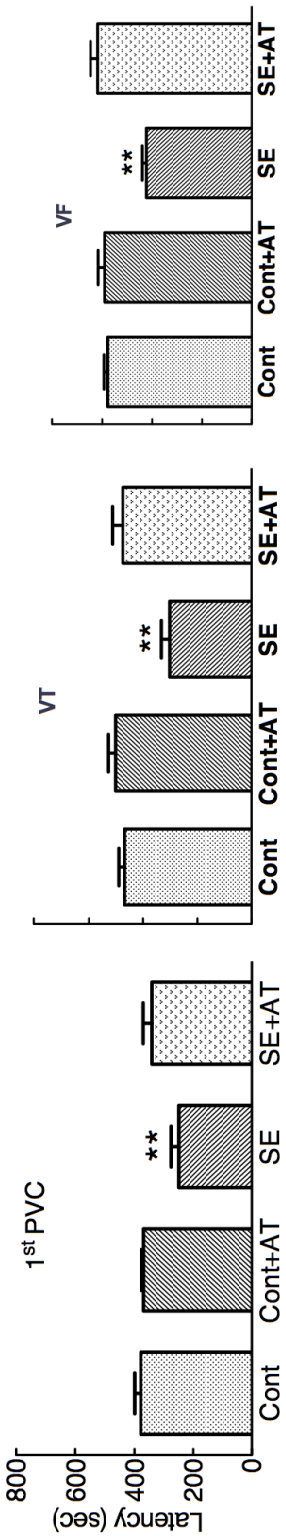


Figure 2.4. Corrected QT interval (Panel A; QTc) and QTc dispersion (Panel B; QTcd) measured 12-14 days following vehicle-treated control (Cont; n=7), atenolol treated Cont (Cont+AT; n=7), vehicle-treated SE (n=8) and atenolol treated SE (SE+AT; n=7) rats. **$p<0.01$ compared to all other groups.

Figure 2.5. Latency from the initiation of aconitine administration to the 1st premature ventricular contraction (1st PVC), ventricular tachycardia (VT), and ventricular fibrillation (VF) measured 12-14 following vehicle-treated control (Cont; n=4), atenolol treated Cont (Cont+AT; n=4), vehicle-treated SE (n=6) and atenolol treated SE (SE+AT; n=5) rats. **p<0.01 compared to all other groups.


1st PVC
Latency (sec)
800
600
400
200
0
Cont
Cont+AT
SE
SE+AT
**
VT
Cont
Cont+AT
SE
SE+AT
**
VF
Cont
Cont+AT
SE
SE+AT
**

arrhythmias observed in SE animals 12-14 days later. These data demonstrate that ß-1 receptor blockade, which inhibits SymNS effects on the heart, provides cardiac protection from lethal arrhythmias following SE.

Cerebral Damage

Representative confocal photomicrographs of Fluoro-Jade B-stained hippocampal sections of Cont and SE animals treated with atenolol or vehicle are shown if Fig. 2.6 (Panel A). The mean number of Fluoro-Jade B positive neurons/section are summarized in Fig. 2.6 (Panel B). Results demonstrated a significant and similar increase in damaged neurons in both SE and SE + AT treated animals when compared to Cont and Cont + AT treated rats, which has no positive stained neurons. These data indicate that atenolol treatment during SE did not alter seizure-related cerebral damage.

Discussion

These data demonstrate that cardiac therapy during prolonged seizure activity with a ß-1 adrenergic antagonist limited sympathetically mediated tachycardia, SE-induced cardiac damage, and arrhythmogenic cardiac disturbances, and normalized susceptibility to experimentally induced ventricular arrhythmias for up to 10-12 days following seizure cessation. Moreover, cardiac protection with atenolol resulted from SymNS blockade

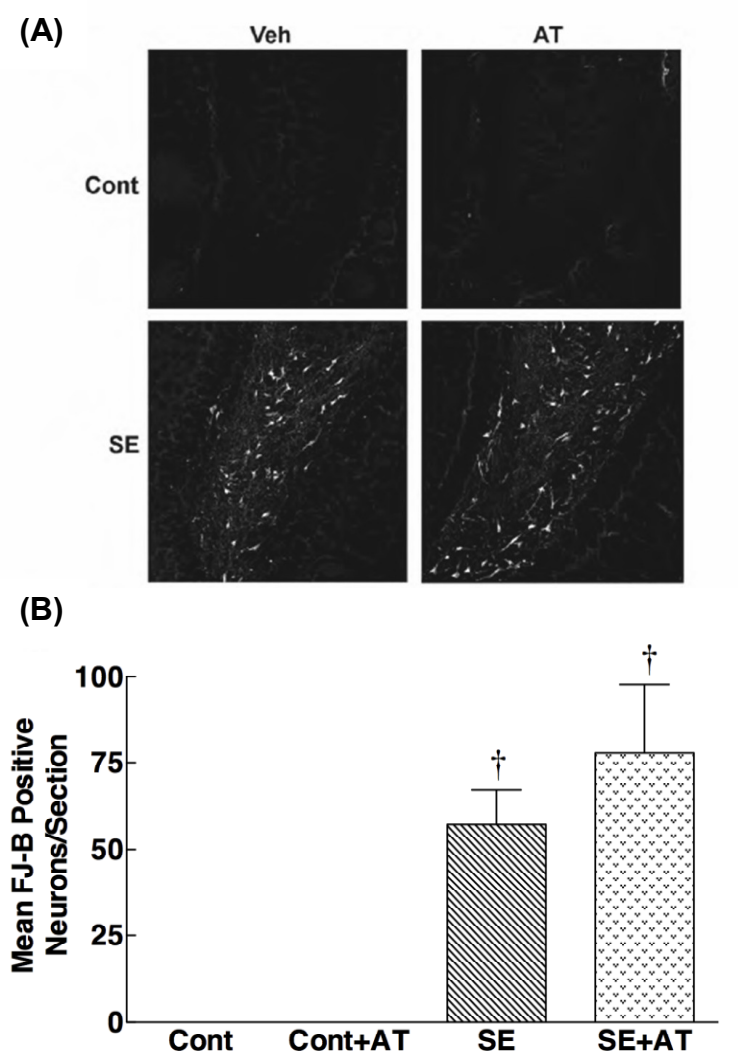


Figure 2.6. Representative confocal photomicrographs (Panel A) of positive Fluoro-Jade B stained neurons in the dentate gyrus of the hippocampus in control (Cont) and rats undergoing status epilepticus (SE) treated with either vehicle (Veh) or atenolol (AT). Mean number of positive Fluoro Jade B stained neurons per hippocampal section in vehicle-treated control (Cont; n=4), atenolol treated Cont (Cont+AT; n=4), vehicle-treated SE (n=6) and atenolol-treated SE (SE+AT; n=5) rats. †$p<0.01$ compared to Cont and Cont+AT.

and not by reduced seizure intensity, as brain damage and motor seizure activity was equivalent in all SE animals. However, this needs to be further supported by video or EEG recordings to quantitated seizure severity and duration. These results support the proposal that intense activation of the SymNS during SE causes overstimulation of ß-1 adrenoceptors on the heart and induces acute cardiac damage, altered cardiac electrophysiology, and increased susceptibility to ventricular arrhythmias.

Prolonged elevation of HR and BP demonstrates activation of the SymNS in these experiments during 90 min of continuous seizure activity. These findings are well supported by both clinical and animal research (Goodman, et al. 1990, Johnston, et al. 1997, Sakamoto, et al. 2008, Shimizu, et al. 2008). In clinical reports, SE induces reversible cardiac damage (Aminoff and Simon 1980, Earnest, et al. 1992, Legriel, et al. 2008, Manno, et al. 2005) and increases risk of lethal arrhythmias in the weeks following seizure cessation (Earnest, et al. 1992, Fountain 2000, Hall 1983, Kumar, et al. 2005, Lathers and Schraeder 2002, Scorza, et al. 2008). Although detrimental cardiac effects were similar to those already observed in patients and animals, these experiments also demonstrate that cardioprotective therapy with a ß-1 receptor blockade prevents SE-induced cardiac deficits. Moreover, these results support the proposal that the mechanism of SE-induced adverse cardiac effects is sympathetically mediated, and suggest a route for beneficial therapy that may reduce mortality.

The QT interval represents the total time for ventricular depolarization and repolarization to occur and is an index of action potential duration (APD). Prolonged QTc and increased QTcd are both indicators of increased arrhythmogenic cardiac electrical activity and thereby are prognostic predictors of increased risk of ventricular arrhythmias that cause sudden death. The present experiments demonstrate that SE produced both prolonged QTc and increased QTcd in rat hearts for at least 12 days following seizure cessation. Moreover, evidence of SymNS mediated alterations to APD during SE, is demonstrate by treatment during seizure with atenolol to prevent ß-1 receptor induced tachycardia, which completely ablated APD prolongation and normalized QTc and QTcd to Cont values. One mechanism that alters QT interval and increases susceptibility to sudden cardiac death is myocardial ion channel remodeling. Cardiac myocyte ion channel remodeling, due to SymNS activation, mimics cardiac ion channelopathies that result from primary genetic mutation or acquired inactivation to ion subunits and interacting proteins—for example, loss of function in potassium channels in long QT syndrome (Schimpf, et al. 2009, Ueda, et al. 2008, Vatta, et al. 2006, Wu, et al. 2008). Indeed, evidence of diminished Kv4.2 potassium channel expression has been demonstrated in rat myocytes at 1 and 2 weeks following SE (Bealer, et al. 2010). Taken together, these results suggest a candidate mechanism by which protracted seizures increase risk of sudden cardiac death in patients through increased cardiac exposure to an arrhythmogenic

facilitator, via ß-adrenergic stimulation by catecholamines, and an acquired electrical substrate, via myocyte ion channel remodeling.

The mechanism by which SymNS hyperactivation produces arrhythmogenic myocardial damage, remodeling and altered cardiac function is not understood. In SE, prolonged seizures induce positive inotropic and chronotropic responses on myocardial function, as indicated by increased HR and dP/dt (Goodman, et al. 1990, Johnston, et al. 1997, Sakamoto, et al. 2008). In addition, seizure activity increases BP, and thereby afterload, which can further stress the heart (Kloner, et al. 2001, Kloner and Jennings 2001a, Kloner and Jennings 2001b). In these experiments, cardiac therapy with a ß-1 receptor antagonist, which limits seizure-induced tachycardia but not afterload, provided protection from neurogenically mediated cardiac damage, alteration in electrophysiology, and normalized susceptibility to arrhythmias. These results suggest that increased afterload alone is not contributing to cardiac stunning; however, the effects of a catecholamine-induced chronotoropic response without the altered pressor response are unknown.

Brain damage has been previously reported in lithium pilocarpine induced SE (Druga, et al. 2010, Nairismagi, et al. 2006, Poirier, et al. 2000). There are likely several mechanisms mediating CNS damage during seizure activity including acute metabolic disturbances, hyperthermia, hypoxia, stroke, head trauma, toxicity, nerve gas exposure, and CNS infection

(Lowenstein and Alldredge 1998). Consequently, it was unknown if preventing SE-related tachycardia during motor seizure activity would exacerbate CNS damage by altering the pressor response and limiting cerebral perfusion. However, these data demonstrated that when HR was maintained at preseizure levels, the pressor response was not significantly altered and BP remained elevated in all SE animals. Moreover, evaluation of CNS damage with Fluoro-Jade B demonstrated that atenolol administration during SE did not significantly alter hippocampal damage compared to vehicle-treated SE animals. These data demonstrate that cardiac protective therapy with atenolol, which blocks sympathetically mediated tachycardia, does not increase risk nor provide protection from CNS neuronal death or damage as a result of altered or diminished perfusion pressure.

Cardiac protection observed in these studies is attributed to peripheral antagonistic effects of atenolol and not alteration in seizure activity. Even though ß-adrenergic receptor antagonists (Nakamura, et al. 2008, Raju, et al. 1998) that cross the blood brain barrier alter seizure threshold (De Sarro, et al. 2002, Luchowska, et al. 2002, Luchowska, et al. 2001), atenolol was selected since it does not readily penetrate the blood brain barrier and thereby does not alter seizure threshold or seizure frequency (De Sarro, et al. 2002, Luchowska, et al. 2002). These data indicate that atenolol treatment during SE did not alter seizure-related cerebral damage or SE intensity. While atenolol did prevent tachycardia during SE it did not prevent increases

in MAP, which suggest that SymNS activity was maintained during seizure activity and that the mechanisms of cardiac protection is primarily due to ß-1 blockade on cardiac myocytes and not a reduced central effect. However, from a clinical treatment paradigm administration of a ß-adrenergic antagonist, which does cross the blood brain barrier, may potentially provide both cardiac and cerebral protection during seizure activity.

These data are consistent with the proposal that SE causes a stress cardiomyopathy that is characterized by increases in plasma cTnI, altered cardiac electrical activity, and a reduction in cardiac function—all of which increase susceptibility to ventricular arrhythmias and sudden death. However, to determine if SE produces neurogenically mediated cardiac stunning, cardiac hemodynamic needed to be evaluated (see Chapter 3). Moreover, these data suggest a prolonged period of cardiac dysfunction following SE in rats, which is consistent with clinical reports that lethal arrhythmias contribute to mortality within 30 days of the episode. Further, these experiments suggest that cardiac therapy during seizure activity may act to improve recovery and decrease mortality associated with SE.

References

Aminoff MJ, Simon RP. (1980) Status epilepticus. Causes, clinical features and consequences in 98 patients. *Am J Med* 69:657-666.

Antman EM. (2002) Decision making with cardiac troponin tests. *N Engl J Med* 346:2079-2082.

Bealer SL, Little JG, Metcalf CS, Brewster AL, Anderson AE. (2010) Autonomic and cellular mechanisms mediating detrimental cardiac effects of status epilepticus. *Epilepsy Res* 91:66-73.

Chen L, Wang L, Xu B, Ni G, Yu L, Han B, Yu X, Wang K, Lai Y, Zhou S, Zhu Q. (2009) Mechanisms of alpha1-adrenoceptor mediated QT prolongation in the diabetic rat heart. *Life Sci.* 84:250-256.

Chugh SS, Reiner K, Singh T, Uy-Evanado, A., Socoteanu C, Peters D, Mariani R, Gunson K, Jui J. (2009) Determinants of prolonged QT interval and their contribution to sudden death risk in coronary artery disease: The Oregon sudden unexpected death study. *Circulation* 119:663-670.

da Silva Costa EC, Concalves AA, Areas MA, Morgabel RGB. (2008) Effects of meformin on QT and QTc interval dispersion in diabetic rats. *Arq. Bras. Cardiol.* 90:232-238.

Darbar D, Luck J, Davidson N, Pringle T, Main G, McNeill G, Struthers AD. (1996) Sensitivity and specificity of QTc dispersion for identification of risk of cardiac death in patients with peripheral vascular disease. *BMJ* 312:874-878.

de Bruyne MC, Hoes AW, Kors JA, Hofman A, van Bemmel JH, Grobbee DE. (1998) QTc dispersion predicts cardiac mrotality in the elderly: The Rotterdam study. *Circulation* 97:467-472.

De Sarro G, Di Paola ED, Ferreri G, De Sarro A, Fishcher W. (2002) Influence of some beta-adrenoceptor antagonists on the anticonvulsant protency of antiepileptic drugs against audiogenic seizures in DBA/2 mice. *Eur. J. Pharmacol.* 442:205-213.

Druga R, Mares P, Kubova H. (2010) Time course of neuronal damage in the hippocampus following lithium-pilocarpine status epilepticus in 12-day-old rats. *Brain Res* 1355:174-179.

Earnest MP, Thomas GE, Eden RA, Hossack KF. (1992) The sudden unexplained death syndrome in epilepsy: demographic, clinical, and postmortem features. *Epilepsia* 33:310-316.

Fountain NB. (2000) Status epilepticus: risk factors and complications. *Epilepsia* 41 Suppl 2:S23-30.

Glien M, Brandt C, Potschka H, Voigt H, Ebert U, Loscher W. (2001) Repeated low-dose treatment of rats with pilocarpine: low mortality but high proportion of rats developing epilepsy. *Epilepsy Res* 46:111-119.

Goodman JH, Homan RW, Crawford IL. (1990) Kindled seizures elevate blood pressure and induce cardiac arrhythmias. *Epilepsia* 31:489-495.

Grippo AJ, Santos CM, Johnson RF, Beltz TG, Martins JB, Felder RB, Johnson AK. (2004) Increased susceptibility to ventricular arrhythmias in a rodent model of experimental depression. *Am J Physiol Heart Circ Physiol* 286:H619-626.

Hall S. (1983) Status epilepticus. *Am Fam Physician* 28:117-121.

Johnston SC, Siedenberg R, Min JK, Jerome EH, Laxer KD. (1997) Central apnea and acute cardiac ischemia in a sheep model of epileptic sudden death. *Ann Neurol* 42:588-594.

Klekot AA. (2006) Antiarrhythmic activity of a membrane-protecting agent Sal'magin in rats with aconitine-induced arrhythmias. *Bull. Exp. Biol. Med.* 142:209-211.

Kloner RA, Arimie RB, Kay GL, Cannom D, Matthews R, Bhandari A, Shook T, Pollick C, Burstein S. (2001) Evidence for stunned myocardium in humans: a 2001 update. *Coron Artery Dis* 12:349-356.

Kloner RA, Jennings RB. (2001a) Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: part 1. *Circulation* 104:2981-2989.

Kloner RA, Jennings RB. (2001b) Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: part 2. *Circulation* 104:3158-3167.

Kulkarni SK, George B. (1995) Lithium-pilocarpine neurotoxicity: a potential model of status epilepticus. *Methods Find. Exp. Clin. Pharamacol.* 17:551-567.

Kumar MA, Urrutia VC, Thomas CE, Abou-Khaled KJ, Schwartzman RJ. (2005) The syndrome of irreversible acidosis after prolonged propofol infusion. *Neurocrit Care* 3:257-259.

Lathers CM, Schraeder PL. (2002) Clinical pharmacology: drugs as a benefit and/or risk in sudden unexpected death in epilepsy? *J Clin Pharmacol* 42:123-136.

Legriel S, Bruneel F, Dalle L, Appere-de-Vecchi C, Georges JL, Abbosh N, Henry-Lagarrigue M, Revault D'Allonnes L, Ben Mokhtar H, Audibert J, Guezennec P, Troche G, Bedos JP. (2008) Recurrent takotsubo cardiomyopathy triggered by convulsive status epilepticus. *Neurocrit Care* 9:118-121.

Lowenstein DH, Alldredge BK. (1998) Status epilepticus. *N Engl J Med* 338:970-976.

Luchowska E, Luchowska P, Wielosz M, Kleinrok Z, Czuczwar SJ, Urbanska EM. (2002) Propranolol and metopropolol enhance the anticonvulsant action of valproate and diazepam against maximal electroshock. *Pharmacol. Biochem. Behav.* 71:223-231.

Luchowska E, Luchowska P, Wielosz M, Kleinrok Z, Urbanska EM. (2001) beta-adrenoceptor blockade enhances the anticonvulsant effect of glutamate receptor antagonists against maximal electroshock. *Eur. J. Pharmacol.* 431:209-214.

Manno EM, Pfeifer EA, Cascino GD, Noe KH, Wijdicks EF. (2005) Cardiac pathology in status epilepticus. *Ann Neurol* 58:954-957.

Metcalf CS, Poelzing S, Little JG, Bealer SL. (2009) Status epilepticus induces cardiac myofilament damage and increased susceptibility to arrhythmias in rats. *Am J Physiol Heart Circ Physiol* 297:H2120-2127.

Nairismagi J, Pitkanen A, Kettunen MI, Kauppinen RA, Kubova H. (2006) Status epilepticus in 12-day-old rats leads to temporal lobe neurodegeneration and volume reduction: a histologic and MRI study. *Epilepsia* 47:479-488.

Nakamura T, Oda Y, Takahashi R, Tanaka K, Hase I, Asada A. (2008) Propranolol increases the threshold for lidocaine-induced convulsions in awake rats: a direct effect on the brain. *Anesth. Analg.* 106:1450-1455.

O'Brien PJ, Smith DE, Knechtel TJ, Marchak MA, Pruimboom-Brees I, Brees DJ, Spratt DP, Archer FJ, Butler P, Potter AN, Provost JP, Richard J, Snyder PA, Reagan WJ. (2006) Cardiac troponin I is a sensitive, specific biomarker of cardiac injury in laboratory animals. *Lab Anim* 40:153-171.

Poirier JL, Capek R, De Koninck Y. (2000) Differential progression of Dark Neuron and Fluoro-Jade labelling in the rat hippocampus following pilocarpine-induced status epilepticus. *Neuroscience* 97:59-68.

Racine RJ. (1972) Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 32:281-294.

Raju SS, Gopalakrishna HN, Venkatadri N. (1998) Effect of propranolol and nifedipine on maximal electroshock-induced seizures in mice: individually and in combination. *Pharmacol. Res.* 38:449-452.

Sakamoto K, Saito T, Orman R, Koizumi K, Lazar J, Salciccioli L, Stewart M. (2008) Autonomic consequences of kainic acid-induced limbic cortical seizures in rats: Peripheral autonomic nerve activity, acute cardiovascular changes, and death. *Epilepsia*:1-15.

Sarko J, Pollack CV. (2002) Cardiac troponins. *J. Emergency Medicine* 23:57-65.

Schimpf R, Veltmann C, Wolpert C, Borggrefe M. (2009) Channelopathies: Brugada syndrome, long QT syndrome, and CPVT. *Herz* 34:281-288.

Scorza FA, Cysneiros RM, Arida RM, Scorza CA, de Almeida AC, Schmidt B, Cavalheiro EA. (2008) Adult hippocampal neurogenesis and sudden unexpected death in epilepsy: reality or just an attractive history? *Med Hypotheses* 71:914-922.

Shimizu M, Kagawa A, Takano T, Masai H, Miwa Y. (2008) Neurogenic stunned myocardium associated with status epilepticus and postictal catecholamine surge. *Intern. Med.* 47:269-273.

Shu H, Yi-Ming W, Xu LP, Miao CY, Su DF. (2004) Increased susceptibility of ventricular arrhythmias to aconitine in anaesthetized rats is attributed to the inhibition of baroreflex. *Clin Exp Pharmacol Physiol* 31:249-253.

Takahara A, Uneyama H, Sasaki N, Ueda H, Dohmoto H, Shoji M, Hara Y, Nakaya H, Yoshimoto R. (1999) Effects of AH-1058, a new antiarrhythmic drug, on experimental arrhythmias and cardiac membrane currents. *J Cardiovasc Pharmacol* 33:625-632.

Ueda K, Valdivia C, Medeiros-Domingo A, Tester DJ, Vatta M, Farrugia G, Ackerman MJ, Makielski JC. (2008) Syntrophin mutation associated with long QT syndrome through activation of the nNOS-SCN5A macromolecular complex. *Proc. Natl. Acad. Sci.* 105:9355-9360.

Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, Tester DJ, Balijepalli RC, Foel JD, Li Z, Kamp TJ, Towbin JA. (2006) Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. *Circulation* 114:2104-2112.

Volk T, Nguyen TH, Schultz JH, Faulhaber J, Ehmke H. (2001) Regional alterations of repolarizing K+ currents among the left ventricular free wall of rats with ascending aortic stenosis. *J Physiol* 530:443-455.

Wu G, Ai T, Kim JJ, Mohapatra B, Xi Y, Li Z, Abbasi S, Purevjav E, Samani K, Ackerman MJ, Qi M, Moss AJ, Shimizu W, Towbin JA, Cheng J, Vatta M. (2008) alpha-1-syntrophin mutation and the long-QT syndrome: a disease of sodium channel disruption. *Circ Arrhythm Electrophysiol* 1:193-201.

CHAPTER 3

ß-1 BLOCKER THERAPY INHIBITS STATUS EPILEPTICUS INDUCED CARDIAC STUNNING

Introduction

The precise mechanisms producing myocardial stunning following SE have not been determined in humans. SE has been proposed to produce stunning through a neurogenic mechanism in which a sudden and prolonged activation of the SymNS and hypersecretion of catecholamines act to over-stimulate ß-adrenergic receptors on cardiac muscle (Sakamoto, et al. 2008, Shimizu, et al. 2008, Simon 1985). Adrenoceptor overstimulation can produce cardiac dysfunction characterized by subtle myocyte damage, cardiac conduction abnormalities, increased susceptibility to arrhythmias, and left ventricular (LV) dysfunction (Bolli and Marban 1999, Legriel, et al. 2008b, Lemke, et al. 2008, Manno, et al. 2005, Metcalf, et al. 2009a, Pomblum, et al. 2010, Walton, et al. 1995). While the first of those three characteristics has been reported to occur as a result of SE in rats (Bealer, et al. 2010b, Metcalf, et al. 2009a), LV dysfunction and the protective benefits of ß-1 receptor

antagonist therapy on cardiac function have not been previously demonstrated in animal models of SE.

Therefore, the present experiments were designed to determine if LV dysfunction, which mimics myocardial stunning in humans, occurs in a rat model of self-sustaining limbic status epilepticus (SSLSE) in rats. Further, these studies were designed to demonstrate that administration of a ß-1 receptor antagonist prevents cardiac dysfunction caused by SE-induced sympathetic activation. Twenty-four hours following SE, cardiac function in rats was determined by measuring cardiac output (CO) and recording LV pulse pressure (LVP), mean arterial pulse pressure (MAP), and ECG activity. These recordings were further evaluated for HR, QTc, the first derivative of the maximum rate of LV pressure development over time (LVP dP/dt max), which is a measure of LV contractility, and the first derivative of the minimum rate of LV pressure development over time (LVP dP/dt min), which is a measure of LV relaxation.

Methods

Animals

Male Sprague-Dawley rats (175-225 g) were purchased from a commercial supplier (Charles River, Wilmington, MA). These rats had *ad libitum* access to standard laboratory rat chow and water, and were housed

in animal quarters maintained at 23°C and a 12hrs:12hrs, light:dark cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Utah.

SSLSE Protocol

Rats were implanted with an electrode in the left amygdala, which served as both a means of electrical stimulation to induce SSLSE and recording electroencephalographs (EEG). To implant electrodes, rats were anesthetized (Avertin, 300mg/kg, ip) and their heads shaved. They were then placed into a stereotaxic frame. A 2-3 cm midline incision was made to expose the skull. A small burr hole was made in the skull directly above the amygdala. Adjacent to the hole, three small plastic anchor screws were inserted into the skull. A bipolar stimulating electrode (9mm length, with 1 mm gap; Plastics1 Inc.) was stereotaxically implanted in the lateral nucleus of the left amygdala (anterior-posterior -3.6, medial-later 5.0, dorsal-ventral -6.5, from bregma and the dura) through the hole and fixed into place with dental acrylic. Once the dental acrylic had hardened the skin was closed with wound clips and an antibiotic ointment was applied. Finally, a cap (Plastics 1 Inc.) was placed on the electrode to keep it clean. After all surgeries, animals were administered Baytril (antibiotic) and Rimadyl (NSAID) and returned to their home cages. Rats were allowed to recover for a minimum of 12-14 days before SSLSE was induced.

SSLSE Induction Protocol

After recovery from electrode implantation, rats were connected to stimulating (IsoMax-100 Biphasic Stimulus Isolator) and EEG recording equipment (Powerlab 2-20; ADInstruments). In animals undergoing SSLSE, an electronic switch (Grass Instruments) was used to alternate between stimulation and EEG recording modes. Stimulations consisted of 40 min of a 100 msec train of 1 msec biphasic square wave pulses at 600 μA, delivered at 60 Hz every 0.5 sec. Electrographic seizures were evaluated from the EEG. Motor seizure activity was visually monitored during the stimulation period and quantified using the Racine scale (Racine 1972). Motor seizures typically began intermittently within the first few minutes of stimulation, and progressed in severity and duration until the seizures became persistent and self-sustaining. After 40 min the stimulation was stopped and EEGs were collected. Successful SSLSE induction was ascertained by monitoring spontaneous motor seizure behavior and recurrent, spontaneous spike wave activity in EEGs (Nissinen, et al. 2000). Following 90 min of continuous SSLSE, animals were administered valproic acid (VPA; 400 mg/kg ip; Sigma, St. Louis, MO) to terminate seizure activity. All animals were then returned to their home cages, where food and water ingestion were monitored. All animals were supplemented with an injection of 3 mL lactated Ringer's solution (ip) and offered water-softened palatable breakfast cereal (Froot Loops) in addition to normal rat chow. Control (Cont) animals received

identical treatment at concurrent time points, but did not receive electrical stimulation of the amygdala.

Evaluation of Seizure Severity by EEG Activity

EEG activity was evaluated by counting the number of spontaneous spike wave occurrences over a 3 min period during SSLSE at 5, 30, 60, and 90 min after the amygdala stimulation was discontinued.

β-1 Adrenergic Blockade with Atenolol

SSLSE animals were administered atenolol (1 mg/kg; SSLSE+AT) or vehicle (SSLSE+VEH) via tail vein injection immediately prior to the initiation of seizure activity. Additional atenolol was given at 30-35 min intervals to maintain baseline HR during seizures. HR was monitored from ECG recordings. Cont rats received saline injections at similar intervals.

ECG Recording Protocol

ECG electrodes were implanted 24 hrs after SSLSE and at an equivalent time point in Cont rats. Leads were constructed from two insulated silver wires with one end of both wires soldered to a connector. To implant these leads, rats were anesthetized with urethane (1.2 g/kg, ip), and incisions (≈ 10 mm), were made in shaved areas on the upper and lower quadrants of the chest. The exposed tips (≈ 5 mm) of the wires were inserted

through the incisions and sutured into the thoracic muscles. The other ends (connector end) of the wires were attached to a Powerlab 2-20 chart recording unit (ADInstruments). The ECG signals were amplified (50x), filtered (1-1000 Hz), digitized, and recorded prior to and during CO and LV measurements.

CO Determination

Immediately after ECG leads were implanted and while the animals were still under anesthesia, CO was measured by thermodilution using a CO module obtained from ADInstruments. Briefly, the neck was shaved, and an incision (≈ 2-3 cm) was made on the right side of the neck. The right jugular vein and right carotid artery were dissected out, occluded with surgical silk, and the vessels opened. Next, the jugular vein was cannulated with polyethylene tubing (PE-50; filled with heparin, 50 U/ml), which was advanced to just below the right atrium. Next, a thermistor probe was inserted through the carotid artery to the aortic arch. The other end of the probe was connected to the CO module and a Powerlab 2-20 chart recorder (ADInstruments). Following a 5 min calibration period, the temperature indicated by the thermistor probe was recorded continuously. In each animal, several thermodilution curves were generated, by sequential injections of 150 uL room temperature saline into a jugular vein catheter. CO was calculated from the recorded thermodilution curves by Chart software (ADInstruments).

LV and Aortic Pulse Pressure Recordings

After CO measures were collected, aortic and LV pulse pressure parameters were recorded using an arterial catheter and pressure transducer. The previously exposed carotid artery was cannulated with polyethylene tubing (PE-50; filled with heparin, 50 U/ml) and cannula inserted to the level of the aortic arch or into LV. The other end of the catheter was directly connected to a pressure transducer attached to a Powerlab Data Acquisition System (ADInstruments) and Windows computer. Arterial and LV pulse pressures were recorded for 3-5 min at each location. From these recordings, MAP, LVP dP/dt max, LVP dP/dt min were calculated using Chart software (ADInstruments).

Evaluation of QT Intervals

From ECG recordings, QT, QRS and RR interval measurements were obtained. The QT interval is the total time of ventricular depolarization and repolarization, starting at the beginning of the Q wave and terminating when the T-wave returns to the isoelectric value. QRS corresponds to conduction time of the LV. The RR interval is the time between two successive heart beats and was used to determine HR. These measurements were averaged across 10-20 consecutive heart beats. Bazzett's formula was used to correct the QT interval (QTc) for heart rate, $QTc=QT/(RR)^{1/2}$. QTc dispersion (Qtcd) was calculated by subtracting the minimum QTc from the maximum QTc for

each animal. These measurements are well-documented indicators of increased risk for sudden cardiac death (Chugh, et al. 2009, Darbar, et al. 1996, de Bruyne, et al. 1998) and have established procedures for their evaluation in rodents (Chen, et al. 2009, da Silva Costa, et al. 2008, Volk, et al. 2001).

Statistical Analysis

All data are presented as means ±SEM. Differences between two means were evaluated with the Student t-test, and differences among multiple means determined following one-way or multiple-factor analysis of variance for repeated measures. A Newman-Keuls a posteriori test was used to determine differences between individual means following ANOVA. A $p<0.05$ was considered significant.

Results

Seizure Activity

Motor seizure activity during SSLSE was characterized by persistent stage 3-4 Racine scale seizures with randomly occurring stage 5 seizures throughout the 90 min period. Spike wave activity was self-sustained and persisted for 90 min in all SSLSE+VEH and SSLSE+AT animals. There were no significant differences in the number or frequency of spike wave

occurrences between the SSLSE+VEH and SSLSE+AT group (see Fig. 3.1). Administration of VPA abolished all motor seizure activity similarly in both groups.

Cardiac Electrical Activity

Twenty-four hours after SSLSE termination, prolonged QTc and QTcd were measured from ECG recordings and summarized in Fig. 3.2. No significant difference was found in the QRS interval 24 hrs following seizure cessation. Representative data is demonstrated in Fig. 3.3. These data indicated significant prolongation in cardiac action potential duration in all animals that underwent SSLSE and saline injections (SSLSE+VEH; n=6) when compared to control animals (Cont, n=6). Conversely, administration of atenolol to animals during SSLSE (SSLSE+AT, n=6) significantly decreased the QTc interval and QTcd prolongation at 24 hrs when compared to SSLSE+VEH treated animals. However, conduction was still significantly prolonged in SSLSE+AT animals compared to Cont rats. These data are consistent with the hypothesis that SE can alter conduction and thereby cardiac function.

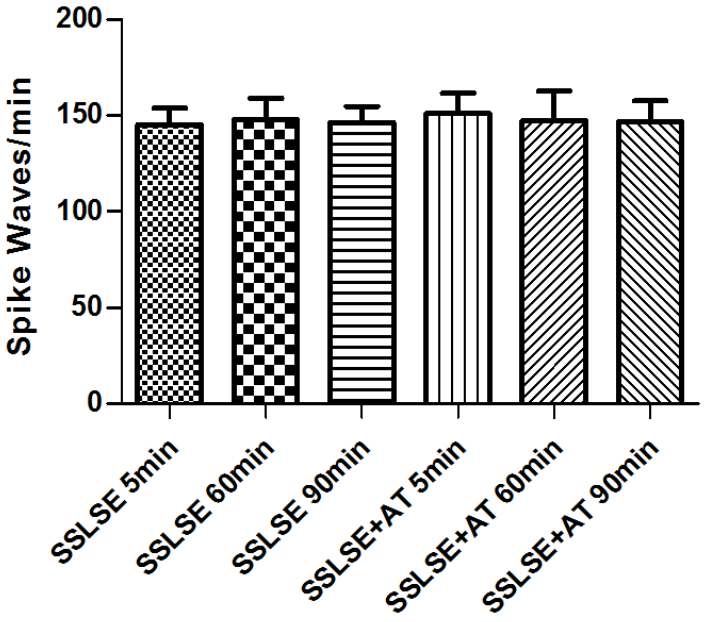


Figure 3.1. These data show the total number of spike wave/min at 5, 60, and 90 min during self-sustained seizure activity in SSLSE and atenolol treated (SSLSE+AT) animals. These data demonstrate that atenolol did not alter seizure intensity in this model.

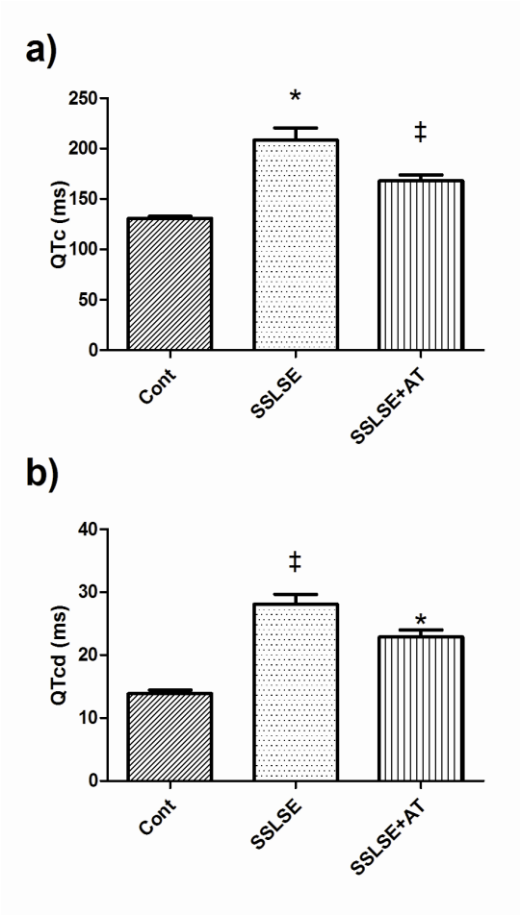
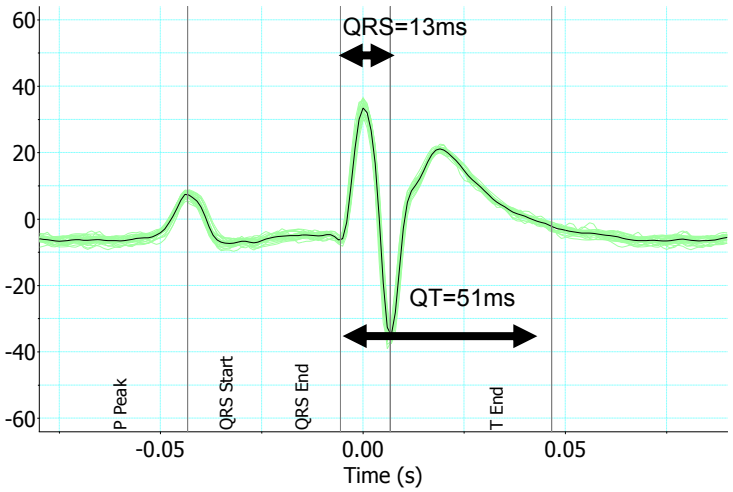


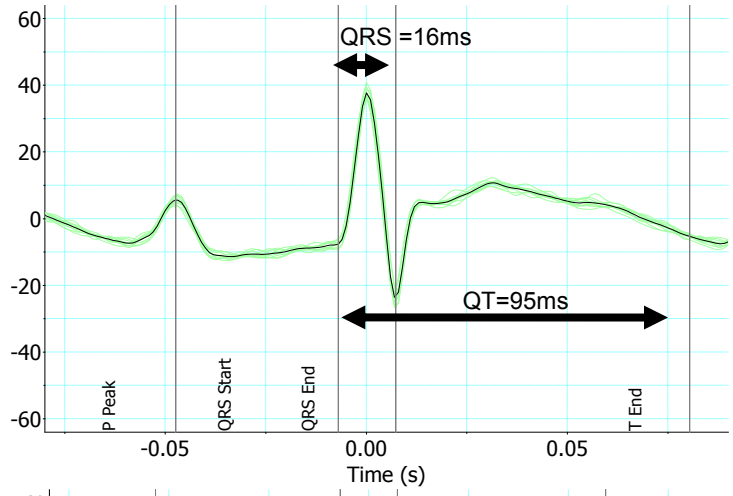
Figure 3.2. QT interval corrected for heart rate (panel a; QTc) and QTc dispersion (panel b; QTcd) in control rats (Cont; n=6) and 24 hrs after rats underwent SSLSE pus saline injections (SSLSE+VEH; n=6) or treatment with atenolol (SSLSE+AT; n=6. *=p<0,05 for SSLSE vs. Cont and SSLSE vs. SSLSE+AT. ‡ =p<0.05 for SSLSE+AT vs. Cont and SSLSE+AT vs. SSLSE.

Figure 3.3. Representative ECGs showing QRS and QT invervals 24 hrs following seizure cessation in SSLSE, SSLSE+AT treated and Cont rats. QT prolongation indicates increase in action potential duration (see Fig. 3.2) and QRS prlongation indicates conduction slowing. Although there was a tendancy for QRS prolongation in the SSLSE rats, it was not significant compared SSLSE+AT or Cont rats.

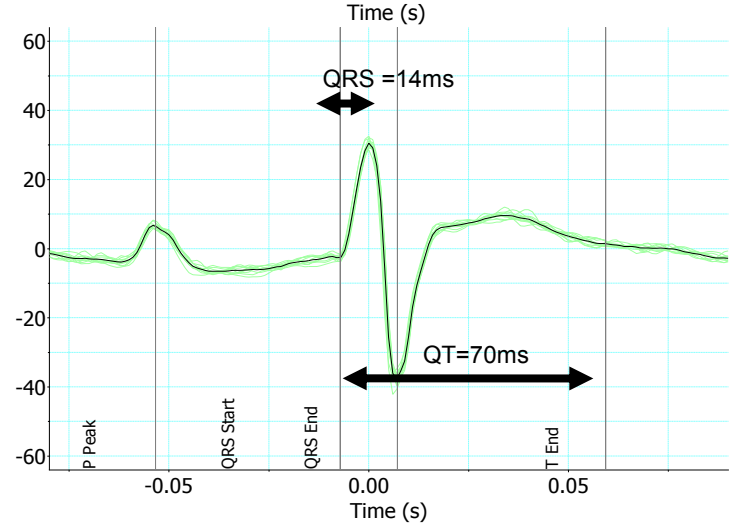
Cont



SSLSE



SSLSE+AT



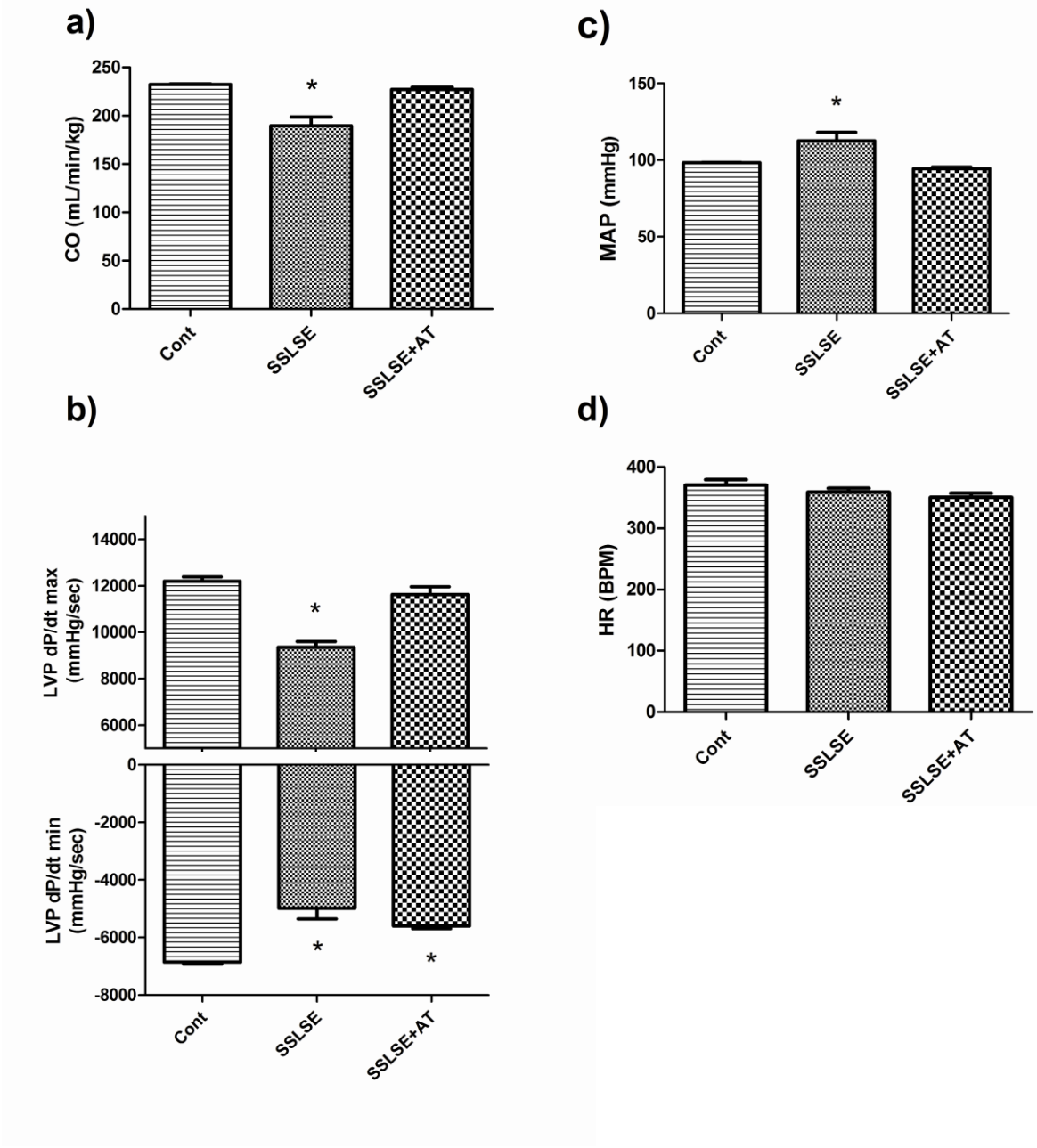
Cardiac Hemodynamic and LV Functional Parameters

Twenty-four hours after SSLSE termination, hemodynamic and functional parameters were determined from carotid artery and LV recordings and are summarized in Fig. 3.4. These data indicate that all animals that underwent SSLSE and saline injections (SSLSE+VEH, n=6) had significant cardiovascular dysfunction including increased MAP, decrease CO, and decreased LVP dP/dt max, when compared to Cont (n=5) animals. Further, treatment of the animals with atenolol during SSLSE (SSLSE+AT, n=5) significantly prevented all of these cardiac alterations when compared to SSLSE+VEH. These data are consistent with the hypothesis that catecholaminergic blockade, with atenolol, inhibits SE-related tachycardia and protects the heart from SE-induced contractile dysfunction. There were no significant differences in HR in any group of animals.

Discussion

These studies are the first to demonstrate diminished cardiac ventricular function in a rat model of SE that is consistent with SE in humans. Further, these studies show that administration of the β-1 receptor antagonist atenolol during SSLSE prevents development of cardiac contractile dysfunctions, which occur within 24 hrs after SE. Together these results provide a potential mechanism and therapeutic strategy for the

Figure 3.4. Cardiac output (a; CO), first derivative of the maximum LV pressure over time (b; LVP dp/dt max) and first derivative of the minimum LV pressure over time (b; LVP dp/dt min), mean arterial pressure (c; MAP), and heart rate (d; HR) were measured in control rats (Cont; n=5) and 24 hrs after rats underwent SSLSE plus saline injections (SSLSE+VEH; n=6) or treatment with atenolol (SSLSE+AT; n=5). *= $p<0.05$ for SSLSE vs. Cont and SSLSE vs. SSLSE +AT.


a)
CO (mL/min/kg)
250
200
150
100
50
0
*
Cont
SSLSE
SSLSE+AT
c)
MAP (mmHg)
150
100
50
0
*
Cont
SSLSE
SSLSE+AT
b)
LVP dP/dt max (mmHg/sec)
14000
12000
10000
8000
6000
*
LVP dP/dt min (mmHg/sec)
0
-2000
-4000
-6000
-8000
*
*
Cont
SSLSE
SSLSE+AT
d)
HR (BPM)
400
300
200
100
0
Cont
SSLSE
SSLSE+AT

prevention of adverse cardiac effects caused by SE, which may reduce mortality risk in patients who experience SE.

These observations further support the proposal that SE results in diminished cardiac function through a neurogenic mechanism in which increased sympathetic tone and high levels of plasma catecholamines induce over-stimulation of the ß-adrenergic receptor on the heart. Thus, without ß-receptor antagonist therapy during SSLSE, prolonged cardiac over-stimulation can produce detrimental cardiac stunning in humans (Bolli 1990, Bolli 1992, Ishiguro and Morgan 2001, Kloner, et al. 2001, Legriel, et al. 2008b, Schulz and Heusch 1995, Shimizu, et al. 2008, Vittone, et al. 2006, Wittstein, et al. 2005). Previous reports have demonstrated that in patients and animals, SE is associated with subtle myofilament damage (Bealer, et al. 2010b), conduction abnormalities (Boggs, et al. 1993), and susceptibility to lethal arrhythmias (Gao, et al. 1995, Legriel, et al. 2008a); however, this is the first report of the effect of SE on diminished LV hemodynamic parameters and changes in cardiac electrophysiology in a non-chemoconvulsive animal model of SE. This suggests that the cardiac damage produced during the Li-pilocarpine model of SE results from seizure activity and not the muscarinic effects of pilocarpine.

Alterations in LV cardiac function were determined by measuring dP/dt during diastole and systole (Borges, et al. 2006, Dowell, et al. 1975), measuring CO, and evaluating cardiac electrophysiology. Results showed

that SSLSE significantly reduced cardiac contraction, prolonged action potential duration, and produced a deficit in LV cardiac pumping efficiency indicative of cardiac stunning within 24 hrs—this was demonstrated by diminished CO, prolonged QTc, and decreased LVP dP/dt max and min, respectively. Further, all of these adverse cardiac effects —except for LVP dP/dt min—were prevented by atenolol administration during SSLSE, which limits catecholamine mediated effects on myocytes. The observed alteration in LV impaired relaxation is a novel finding among diseases contributing to LV dysfunction and correlates well with the observed prolongation in action potential duration. However, the mechanism by which SSLSE induces diminished LVP dP/dt min in both the SSLSE and SSLSE+AT treated animals is not clear, especially given that animals received cardioprotective therapy. Lathers et al. have reported cardiac autonomic nerve centers do not always respond in a predicable manner in animal models of seizures (Lathers and Schraeder 1982, Lathers and Schraeder 1987). It is likely that both blood pressure and cardiac control centers, which are not protected with a selective ß-1 antagonist therapy, are damaged during SSLSE and contribute to altered cardiac function independent of cardiac damage. Further investigation of SE induced alteration during seizure activity on vascular smooth muscle, pressor responses, and sympathoexcitatory brain sites is needed to understand their respective contribution to altered cardiac function and increased mortality. Importantly, diminished cardiac function increases risk factors associated

with mortality (Manno, et al. 2005, Metcalf, et al. 2009a, Metcalf, et al. 2009b). Atenolol therapy may thereby reduce mortality risk through cardiac protection from β-receptor over-stimulation, however additional studies are needed to understand the effects of SE on vascular damage or alterations to cardiac autonomic control centers. Collectively, the current observations combined with previous reports (Bealer, et al. 2010a, Metcalf, et al. 2009a, Metcalf, et al. 2009b) demonstrate that SSLSE produces all of the aforementioned characteristics of neurogenically mediated cardiac stunning within 24 hrs of seizure cessation.

In the SSLSE+AT group, significant changes in QTc and QTcd, which were less than the SSLSE group but more than Cont, were observed. The mechanism that increases QTc and QTcd in SSLSE + AT rats is not clear. It is possible that a residual increase in SymNS drive may persist following concurrent cessation of SSLSE and termination of atenolol administration, which alters cardiac electrophysiology. Alternatively, a catecholamine independent mechanism may alter QTc and QTcd. Regardless of the mechanism, we have previously reported that rats receiving atenolol administration during 90 min of SE did not exhibit any changes in QTc or QTcd at 10 days following seizure cessation (Bealer, et al. 2010b). Therefore, the alterations in QTc and QTcd observed in the atenolol treated animals in the present experiments are likely only acute and quickly recover.

Cardiac protection observed in these studies is attributed to peripheral antagonistic effects of atenolol and not alteration in seizure activity. Even though ß-adrenergic receptor antagonists (Nakamura, et al. 2008, Raju, et al. 1998) that cross the blood brain barrier alter seizure threshold (De Sarro, et al. 2002, Luchowska, et al. 2002, Luchowska, et al. 2001), atenolol was selected since it does not readily penetrate the blood brain barrier and does not alter seizure threshold or seizure frequency (De Sarro, et al. 2002, Luchowska, et al. 2002). These experiments demonstrate that the administration of atenolol did not alter spike wave frequency, initiation or progression for 90 min of SSLSE. Moreover, progressive administration of atenolol did not significantly alter or attenuate either spike wave activity frequency or observed motor seizure behavior during the seizures.

Results of cardiac electrophysiology and hemodynamic data presented in this Chapter are consistent with cardiac alterations found in Chapter 2, which demonstrated cardiac damage during SE, chronic alterations in cardiac action potential duration, and increased susceptibility to arrhythmias 10-14 days following SE. Results presented in Chapter 3 extend these findings and demonstrate that cardiac effects occur acutely. This supports the proposal that SE increases the susceptibility to mortality in the period following seizure cessation (Chin, et al. 2004). Together data from Chapters 2 and 3 demonstrate a contractile dysfunction characterized by neurogenically mediated cardiac damage, altered contractility, and

diminished CO; however to determine if SE results in cardiac stunning, which is defined as reversible contractile dysfunction without gross damage, cardiac recovery needed to be evaluated (see Chapter 4). Further, these data demonstrate pathological substrates that increase susceptibility to arrhythmias, however the mechanism remains unknown (see Chapter 5).

In summary, during SE, there is a sudden and prolonged increase in SymNS activity, which can result in overstimulation of ß-adrenergic receptors on the heart causing subtle myofilament damage (Metcalf, et al. 2009a), arrhythmogenic electrical activity, and cardiac contractile dysfunction—all of which are characteristics of cardiac stunning. Although the exact mechanism is not yet clear, it is proposed that the positive chronotropic and inotropic effects of prolonged exposure to high levels of catecholamines result in tachycardic ischemia. Additionally, catecholamine-induced cardiac dysfunction is indicative of neurogenically mediated cardiac stunning (Ishiguro and Morgan 2001, Wittstein, et al. 2005). Further, our results suggest that during SSLSE, antagonism of ß-1 adrenoceptors can prevent cardiac dysfunctions and thereby may reduce stunned myocardium and decrease mortality-associated risk factors. Finally, these results provide a rationale for cardiac monitoring during and following SE and indicate a therapeutic strategy for cardioprotective treatment in patients.

References

Bealer SL, Little JG, Metcalf CS, Brewster AL, Anderson AE. (2010a) Autonomic and cellular mechanisms mediating detrimental cardiac effects of status epilepticus. *Epilepsy Res*.

Bealer SL, Little JG, Metcalf CS, Brewster AL, Anderson AE. (2010b) Autonomic and cellular mechanisms mediating detrimental cardiac effects of status epilepticus. *Epilepsy Res* 91:66-73.

Boggs JG, Painter JA, DeLorenzo RJ. (1993) Analysis of electrocardiographic changes in status epilepticus. *Epilepsy Res* 14:87-94.

Bolli R. (1990) Mechanisms of myocardial "stunning". *Circulation* 82:173-738.

Bolli R. (1992) Myocardial 'stunning' in man. *Circulation* 86:1671-1691.

Bolli R, Marban E. (1999) Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* 79:609-634.

Borges GR, de Oliveira M, Salgado HC, Fazan R, Jr. (2006) Myocardial performance in conscious streptozotocin diabetic rats. *Cardiovasc Diabetol* 5:26.

Chen L, Wang L, Xu B, Ni G, Yu L, Han B, Yu X, Wang K, Lai Y, Zhou S, Zhu Q. (2009) Mechanisms of alpha1-adrenoceptor mediated QT prolongation in the diabetic rat heart. *Life Sci.* 84:250-256.

Chin RF, Neville BG, Scott RC. (2004) A systematic review of the epidemiology of status epilepticus. *Eur J Neurol* 11:800-810.

Chugh SS, Reiner K, Singh T, Uy-Evanado, A., Socoteanu C, Peters D, Mariani R, Gunson K, Jui J. (2009) Determinants of prolonged QT interval and their contribution to sudden death risk in coronary artery disease: The Oregon sudden unexpected death study. *Circulation* 119:663-670.

da Silva Costa EC, Concalves AA, Areas MA, Morgabel RGB. (2008) Effects of meformin on QT and QTc interval dispersion in diabetic rats. *Arq. Bras. Cardiol.* 90:232-238.

Darbar D, Luck J, Davidson N, Pringle T, Main G, McNeill G, Struthers AD. (1996) Sensitivity and specificity of QTc dispersion for identification of risk of cardiac death in patients with peripheral vascular disease. *BMJ* 312:874-878.

de Bruyne MC, Hoes AW, Kors JA, Hofman A, van Bemmel JH, Grobbee DE. (1998) QTc dispersion predicts cardiac mrotality in the elderly: The Rotterdam study. *Circulation* 97:467-472.

De Sarro G, Di Paola ED, Ferreri G, De Sarro A, Fishcher W. (2002) Influence of some beta-adrenoceptor antagonists on the anticonvulsant protency of antiepileptic drugs against audiogenic seizures in DBA/2 mice. *Eur. J. Pharmacol.* 442:205-213.

Dowell RT, Cutilletta AF, Sodt PC. (1975) Functional evaluation of the rat heart in situ. *J Appl Physiol* 39:1043-1047.

Gao WD, Atar D, Backx PH, Marban E. (1995) Relationship between intracellular calcium and contractile force in stunned myocardium. Direct evidence for decreased myofilament Ca2+ responsiveness and altered diastolic function in intact ventricular muscle. *Circ Res* 76:1036-1048.

Ishiguro Y, Morgan JP. (2001) Effect of endogenous catecholamine on myocardial stunning in a simulated ischemia model. *Fundamental & Clinical Pharmacology* 15:111-116.

Kloner RA, Arimie RB, Kay GL, Cannom D, Matthews R, Bhandari A, Shook T, Pollick C, Burstein S. (2001) Evidence for stunned myocardium in humans: a 2001 update. *Coron Artery Dis* 12:349-356.

Lathers CM, Schraeder PL. (1982) Autonomic dysfunction in epilepsy: characterization of autonomic cardiac neural discharge associated with pentylenetetrazol-induced epileptogenic activity. *Epilepsia* 23:633-647.

Lathers CM, Schraeder PL. (1987) Review of autonomic dysfunction, cardiac arrhythmias, and epileptogenic activity. *J Clin Pharmacol* 27:346-356.

Legriel S, Bruneel F, Dalle L, Apere-de-Vecchi C, Georges JL, Abbosh N, Hernry-Lagarrigue M, Revault D'Allones L, Ben Mokhtar H, Audibert J, Guezennec P, Troche G, Bedos JP. (2008a) Recurrent Takotsubo cardiomyopathy triggered by convulsive status epilepticus. *Neurocrit. Care* Epub.

Legriel S, Bruneel F, Dalle L, Appere-de-Vecchi C, Georges JL, Abbosh N, Henry-Lagarrigue M, Revault D'Allonnes L, Ben Mokhtar H, Audibert J, Guezennec P, Troche G, Bedos JP. (2008b) Recurrent takotsubo cardiomyopathy triggered by convulsive status epilepticus. *Neurocrit Care* 9:118-121.

Lemke DM, Hussain SI, Wolfe TJ, Torbey MA, Lynch JR, Carlin A, Fitzsimmons B-FM, Zaidat OO. (2008) Tako-tsubo cardiomyopathy associated with seizures. *Neurocrit. Care*.

Luchowska E, Luchowska P, Wielosz M, Kleinrok Z, Czuczwar SJ, Urbanska EM. (2002) Propranolol and metopropolol enhance the anticonvulsant action of valproate and diazepam against maximal electroshock. *Pharmacol. Biochem. Behav.* 71:223-231.

Luchowska E, Luchowska P, Wielosz M, Kleinrok Z, Urbanska EM. (2001) beta-adrenoceptor blockade enhances the anticonvulsant effect of glutamate receptor antagonists against maximal electroshock. *Eur. J. Pharmacol.* 431:209-214.

Manno EM, Pfeifer EA, Cascino GD, Noe KH, Wijdicks EF. (2005) Cardiac pathology in status epilepticus. *Ann Neurol* 58:954-957.

Metcalf CS, Poelzing S, Little JG, Bealer SL. (2009a) Status epilepticus induces cardiac myofilament damage and increased susceptibility to arrhythmias in rats. *Am J Physiol Heart Circ Physiol* 297:H2120-2127.

Metcalf CS, Radwanski PB, Bealer SL. (2009b) Status epilepticus produces chronic alterations in cardiac sympathovagal balance. *Epilepsia* 50:747-754.

Nakamura T, Oda Y, Takahashi R, Tanaka K, Hase I, Asada A. (2008) Propranolol increases the threshold for lidocaine-induced convulsions in awake rats: a direct effect on the brain. *Anesth. Analg.* 106:1450-1455.

Nissinen J, Halonen T, Koivisto E, Pitkanen A. (2000) A new model of chronic temporal lobe epilepsy induced by electrical stimulation of the amygdala in rat. *Epilepsy Res* 38:177-205.

Pomblum VJ, Korbmacher B, Cleveland S, Sunderdiek U, Klocke RC, Schipke JD. (2010) Cardiac stunning in the clinic: the full picture. *Interact CardioVasc Thorac Surg* 10:86-91.

Racine RJ. (1972) Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 32:281-294.

Raju SS, Gopalakrishna HN, Venkatadri N. (1998) Effect of propranolol and nifedipine on maximal electroshock-induced seizures in mice: individually and in combination. *Pharmacol. Res.* 38:449-452.

Sakamoto K, Saito T, Orman R, Koizumi K, Lazar J, Salciccioli L, Stewart M. (2008) Autonomic consequences of kainic acid-induced limbic cortical seizures in rats: Peripheral autonomic nerve activity, acute cardiovascular changes, and death. *Epilepsia*:1-15.

Schulz R, Heusch G. (1995) Characterization of hibernating and stunned myocardium. *Eur Heart J* 16 Suppl J:19-25.

Shimizu M, Kagawa A, Takano T, Masai H, Miwa Y. (2008) Neurogenic stunned myocardium associated with status epilepticus and postictal catecholamine surge. *Intern. Med.* 47:269-273.

Simon RP. (1985) Physiologic consequences of status epilepticus. *Epilepsia* 26 Suppl 1:S58-66.

Vittone L, Said M, Mattiazzi A. (2006) beta 2-Adrenergic stimulation is involved in the contractile dysfunction of the stunned heart. *Naunyn Schmiedebergs Arch Pharmacol* 373:60-70.

Volk T, Nguyen TH, Schultz JH, Faulhaber J, Ehmke H. (2001) Regional alterations of repolarizing K+ currents among the left ventricular free wall of rats with ascending aortic stenosis. *J Physiol* 530:443-455.

Walton NY, Rubinstein BK, Treiman DM. (1995) Cardiac hypertrophy secondary to status epilepticus in the rat. *Epilepsy Res* 20:121-124.

Wittstein IS, Thiemann DR, Lima JA, Baughman KL, Schulman SP, Gerstenblith G, Wu KC, Rade JJ, Bivalacqua TJ, Champion HC. (2005) Neurohumoral features of myocardial stunning due to sudden emotional stress. *N Engl J Med* 352:539-548.

CHAPTER 4

MRI IMAGING OF LEFT VENTRICULAR DYSFUNCTION FOLLOWING STATUS EPILEPTICUS AND RECOVERY

Introduction

Prolonged seizure activity is associated with intense activation of the SymNS that produces cardiac damage, tachycardia, hypertrophy, altered cardiac electrical activity, and increased susceptibility to arrhythmias. These cardiomyopathies are indicative of neurogenically mediated cardiac stunning, which is a reversible contractile dysfunction that persists after reperfusion of a short and severe ischemic insult to the heart in which no gross damage has occurred (Bolli and Marban 1999, Pomblum, et al. 2010). In the short term, cardiac stunning produces a contractile dysfunction that increases mortality risk; conversely, the long-term prognosis for stunned myocardium is complete recovery of cardiac function (Jain, et al. 2004). Results in Chapter 3 demonstrate decreased LV function 24 hrs post-SE in animals that is indicative of stunning; however, due to the invasive nature of cardiac catheterization and the use of urethane (from which animals are not allowed to recover), repeated measures over a time course to evaluate cardiac

recovery were not feasible. Thus, to further characterize cardiac deficits and to investigate cardiac recovery following SE we used a noninvasive technique, magnetic resonance imaging (MRI). This noninvasive technique allowed repeated cardiac measurements following SE and during recovery. Further, this technique allowed cardiac myocardium to be viewed for gross cardiac damage acutely and remodeling following 45 days of recovery. We hypothesized that 90 min of SE would produce cardiac effects similar to stunning within 24 hrs, from which rats recover over several weeks. Although the acute cardiac effects have been previously described following SE (discussed in Chapter 3), cardiac recovery following SE termination has not been thoroughly investigated in rats. Cardiac function was measured using functional MRI imaging of the short axis of the left ventricle (LV) at 24 hrs, 10 and 45 days following SE. These images were evaluated for chamber volume, LV ejection fraction, LV stroke volume, myocardial edema, and gross remodeling. Additionally, T2 weighted MRI scans of the brain were collected and evaluated for brain damage and edema over the same period. Together, these results demonstrate recovery of cardiac dysfunction and progress of cerebral edema following 90 min of SE.

Methods

Animals

Male Sprague-Dawley rats (225-250 g) were obtained from a commercial supplier (Charles River, Wilmington, MA) and maintained at 22°C on a 12 hr:12 hr, light:dark schedule. Animals were housed two to three per cage in Plexiglas cages before treatment and individually following SE. Animals were allowed ad libitum access to standard laboratory rat chow and water. The Institutional Animal Care and Use Committee at the University of Utah approved all experimental procedures.

Induction of SE

SE was induced by sequential administration with lithium and pilocarpine, and has been thoroughly described elsewhere (Glien, et al. 2001, Kulkarni and George 1995). Briefly, an injection of lithium (127 mM/kg ip; Sigma, St. Louis, MO) was given 18-24 hrs prior to pretreatment with methyl-scopolamine (2 mg/kg ip, Sigma) for 30 min, followed by administration of pilocarpine (30 mg/kg ip, Sigma) to induce SE. Control (Cont) animals were administered 0.9% saline vehicle in place of pilocarpine. A modified Racine scale (Racine 1972) was used to evaluate seizure activity. Onset of SE was determined by the first grade III or greater seizure followed by more intense and persistent seizures. After 90 min of sustained SE,

valproic acid (VPA; 400 mg/kg ip, Sigma) was administered to terminate motor seizure activity. Cont animals received injections of VPA at similar time points.

Each animal's body weight, eating, and drinking were monitored throughout experiments for up to 45 days. Any hypodypsia and hypophasia were treated with administration of lactated Ringer's solution (3 mL ip) and softened breakfast cereal (Froot Loops) in addition to normal laboratory diet.

MRI Scans

MRI procedures were performed with the assistance of the Small Animal Imaging Facility at the University of Utah. MRI scans were performed to determine changes in indices of heart function in all animals at 24 hrs, 10 and 45 days following SE cessation. To perform MRI protocols, each animal was exposed to 4% isoflurane until an appropriate plane of anesthesia was induced and was maintained with 2-3% isoflurane. Each rat was placed prone in the MRI equipment on a pressure sensor to measure respiration, and a pulse oximeter was attached to the rat's foot to monitor HR and oxygen saturation.

The heart was imaged using a Bruker Biospec 7T/30 cm system operated with Bruker AVANCE II electronics (Bruker BioSpin MRI GmbH, Ettlingen, Germany). A Bruker birdcage quadrature resonator (72 mm inner diameter) was used for signal transmission and reception (Bruker BioSpin

MRI GmbH, Ettlingen, Germany). CINE scans of the heart were acquired using black blood FLASH scan with retrospective gating. The instrument settings were as follows: repetition time (TR) = 31 ms, echo time (TE) = 2.8 ms, number of averages (NEX) = 4, field of view (FOV) = 8 cm x 8 cm, in-plane resolution = 408 x 312 microns, slice thickness = 1.5 mm, number of movie frames = 15. Prospective gating was achieved using SAII monitoring and gating systems (SA Instruments, Stony Brook, NY, USA).

Resultant CINE MRI sequences were used to calculate left ventricular volume throughout the cardiac cycle using freely available software, Segment v1.8 (Segment; http://segment.heiberg.se). The left ventricle was analyzed in a semi-automated manner by an investigator blinded to the treatment groups. Wall thickness was measured at the mid-ventricular level in a short-axis view; papillary muscles were excluded from these measures. Stroke volume was calculated by subtracting the LV end-diastolic volume from LV end-systolic volume. Ejection fraction was calculated by dividing stroke volume by end-diastolic volume.

MRI scans of the brain were also performed to evaluate damage in all animals at 24 hrs, 10 and 45 days following SE cessation. Cerebral spinal fluid and edema appear brighter than white and gray matter in T2-weighted images. Twelve coronal T2-weighted images of the brain were obtained using fast spin echo (FSE) pulse sequence (TR 4 s, TE 30 ms, echo train length 4,

field of view 2.5 x 2.5 cm) with in-plane resolution of 156 x 200 microns, and slice thickness of 2 mm.

Statistical Analysis

All data are presented as means±SEM. Comparison between baseline control measurements and subsequent values were analyzed with a two-way analysis of variance for repeated measures. A Bonferonni posteriori test was used to determine differences in replicate means following ANOVA. A $p<0.05$ was considered significant.

Results

LV Chamber Volume

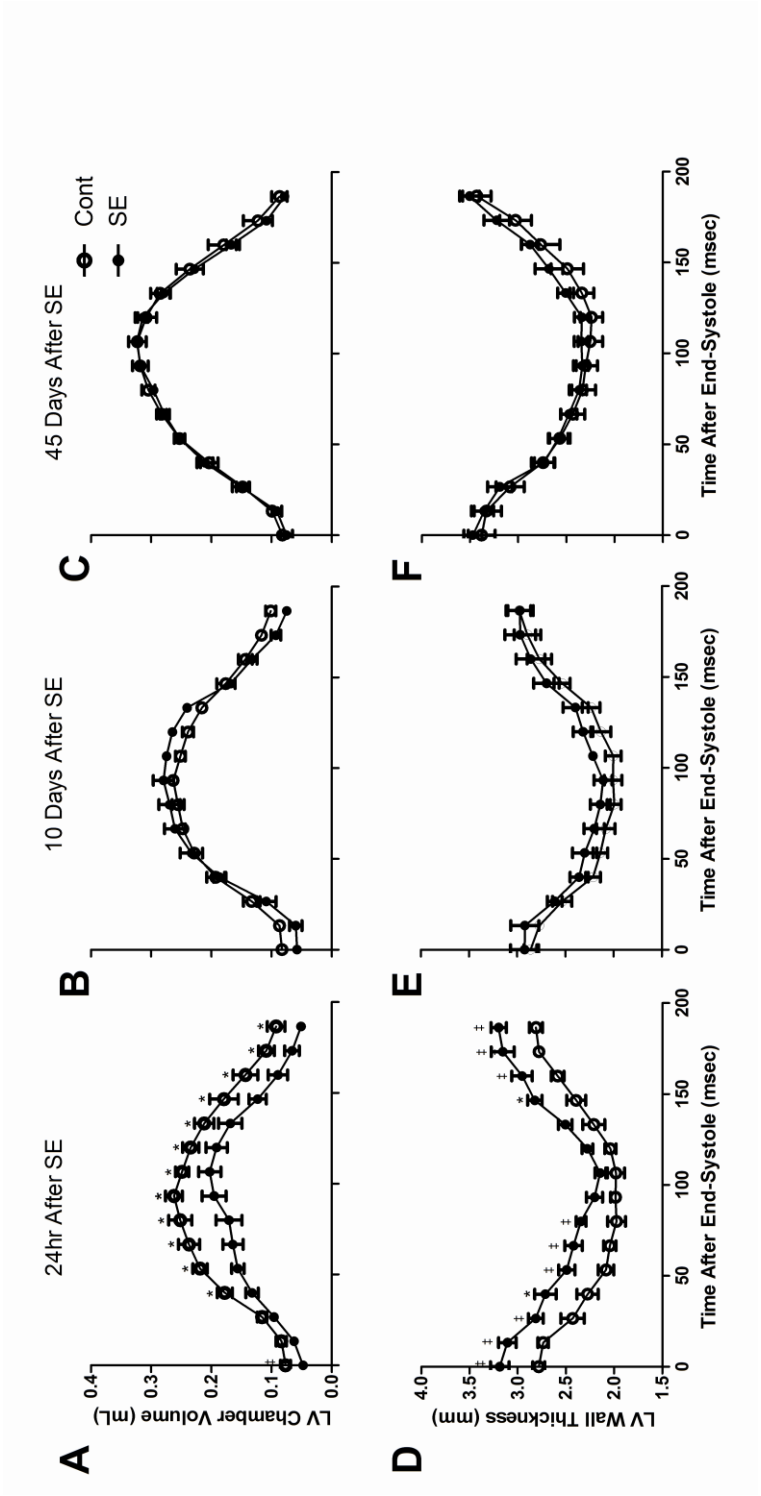
Fig. 4.1A-C summarizes LV chamber volumes measured from MRI scans at 15 time points throughout diastole and systole at baseline and at 24 hrs, 10 and 45 days following 90 min of SE. Within 24 hrs, animals that had undergone SE (24 hrs, n=5) had a significant decrease in LV chamber volume throughout diastole and systole compared to Cont animals (24 hrs, n=5). However, by days 10 and 45 all animals that had undergone SE (10 days, n=3; 45 days, n=3) exhibited a similar chamber volume compared to Cont (10 days, n=3; 45 days, n=3). A plot of LV chamber volume vs. one complete heart beat further demonstrates an impaired lusitropic response within 24 hrs in

animals that underwent SE when compared to Cont animals (see Fig. 4.1A), which recovers within 10 days. These results are consistent with the hypothesis that SE induces a reversible contractile dysfunction characterized by diminished LV stroke work that is indicative of cardiac stunning within 24 hrs in rats. Additionally, these cardiac deficits recover within 10 days and did not produce any apparent chronic alteration in LV function.

LV Wall Thickness

LV wall thickness was measured from MRI scans at 15 time points throughout systole and diastole at baseline and at 24 hrs, 10 and 45 days following 90 min of SE in rats and is summarized in Fig. 4.1D-F. SE produced a significant increase in LV wall thickness within 24 hrs, in all SE animals (24 hrs, n=5; 10 days, n=3; 45 days n=3) throughout diastole and systole when compared to Cont animals (24 hrs, n=5). However, at 10 and 45 days animals that had undergone SE (10 days, n=3; 45 days n=3) exhibited a similar LV wall thickness when compared to Cont (10 days, n=3; 45 days n=3). A plot of LV wall thickness vs. time demonstrates LV hypertrophy within 24 hrs, which recovers in 10 days, in animals that underwent SE when compared to Cont animals (see Fig. 4.1D). These results are consistent with the proposal that SE induces a reversible LV diastolic dysfunction in rats similar to cardiac stunning in humans within 24 hrs following SE cessation. Further, these data demonstrate that SE-induced cardiac

Figure 4.1. These graphs show the LV chamber volume (Panels A, B, and C) and the average wall thickness (Panels D, E, and F) measured from MRI images through diastole and systole starting at end-systole in SE animals (A, n=5; B, n=3; C, n=3) and Cont (A, n=5; B, n=3; C, n=3) at 24 hrs, 10 and 45 days following 90 min of SE. *= $p<0.05$ for SSLSE vs. Cont.


A
24hr After SE
LV Chamber Volume (mL)
0.4
0.3
0.2
0.1
0.0
B
10 Days After SE
C
45 Days After SE
Cont
SE
D
LV Wall Thickness (mm)
4.0
3.5
3.0
2.5
2.0
1.5
E
F
0
50
100
150
200
Time After End-Systole (msec)

dysfunction and hypertrophy is significantly recovered within 10 days and does not produce any apparent chronic alteration in wall thickness.

Stroke Volume and Ejection Fraction

Fig. 4.2 summarizes LV ejection fractions and stroke volumes using LV chamber volumes measured from MRI scans at 15 time points throughout systole and diastole at 24 hrs, 10 and 45 days following 90 min of SE in Cont (24 hrs, n=5; 10 days, n=3; 45 days, n=3) and SE rats (24 hrs, n=5; 10 days, n=3; 45 days, n=3). SE produced a significant increase in LV ejection fraction and a decrease in stroke volume, characterized by a decrease in both end-diastolic volume and stroke volume, in SE animals at 24 and 10 days following seizure cessation. No significant difference between SE and Cont rats was observed by 45 days. These results are consistent with the hypothesis that SE induces both a reversible diastolic dysfunction similar to cardiac stunning in humans following SE cessation. Further, these data demonstrate that SE-induced cardiac dysfunction is significantly recovers and does not produce any apparent chronic cardiac dysfunction.

T2 Weighted Brain Images

Fig. 4.3 shows representative images of T2 weighted coronal images through anterior, medial and posterior sections of the rat hippocampus at

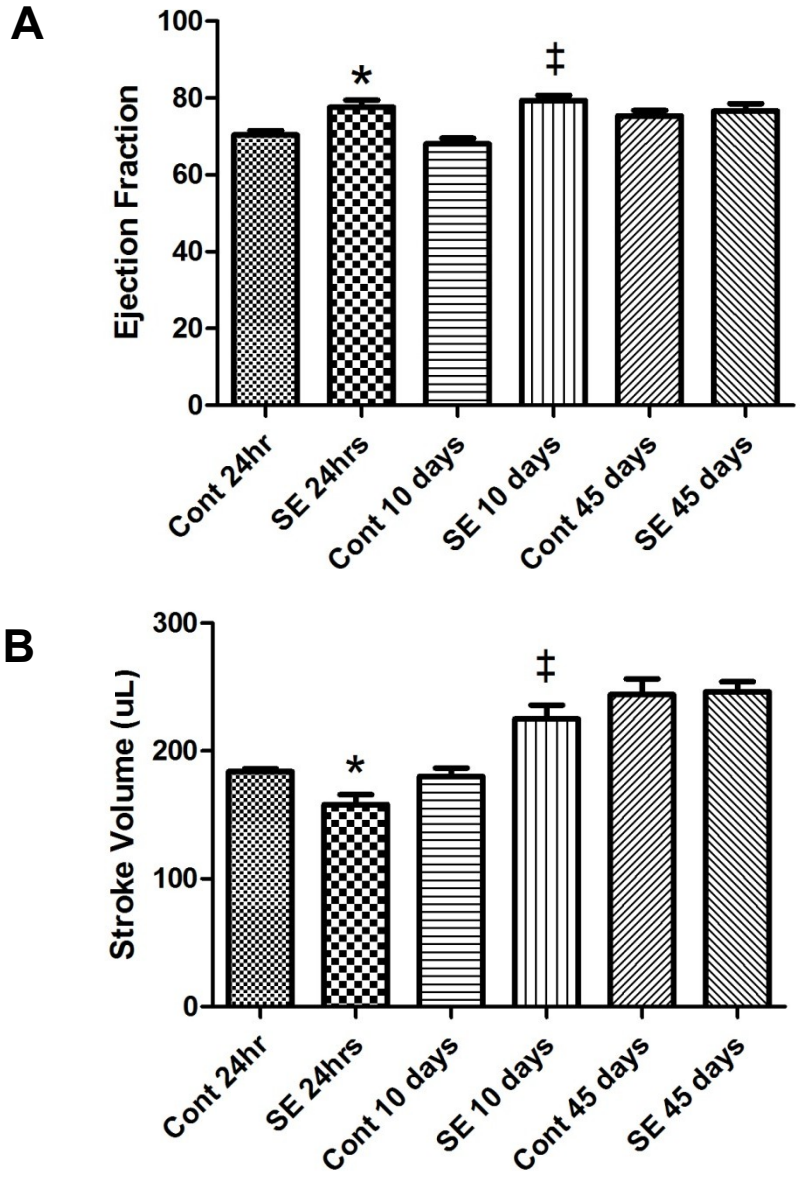


Figure 4.2. Ejection Fraction (Panel A) and LV Stroke Volume (D) measured from MRI images in SE animals (24 hrs, n=5; 10 days, n=3; 45 days, n=3) and Cont (24 hrs, n=5; 10 days, n=3; 45 days, n=3) following 90 min of SE. *= $p<0.05$ for SE 24 hrs vs. Cont 24 hrs. ‡= $p<0.05$ for SE 10 days vs. Cont 10 days.

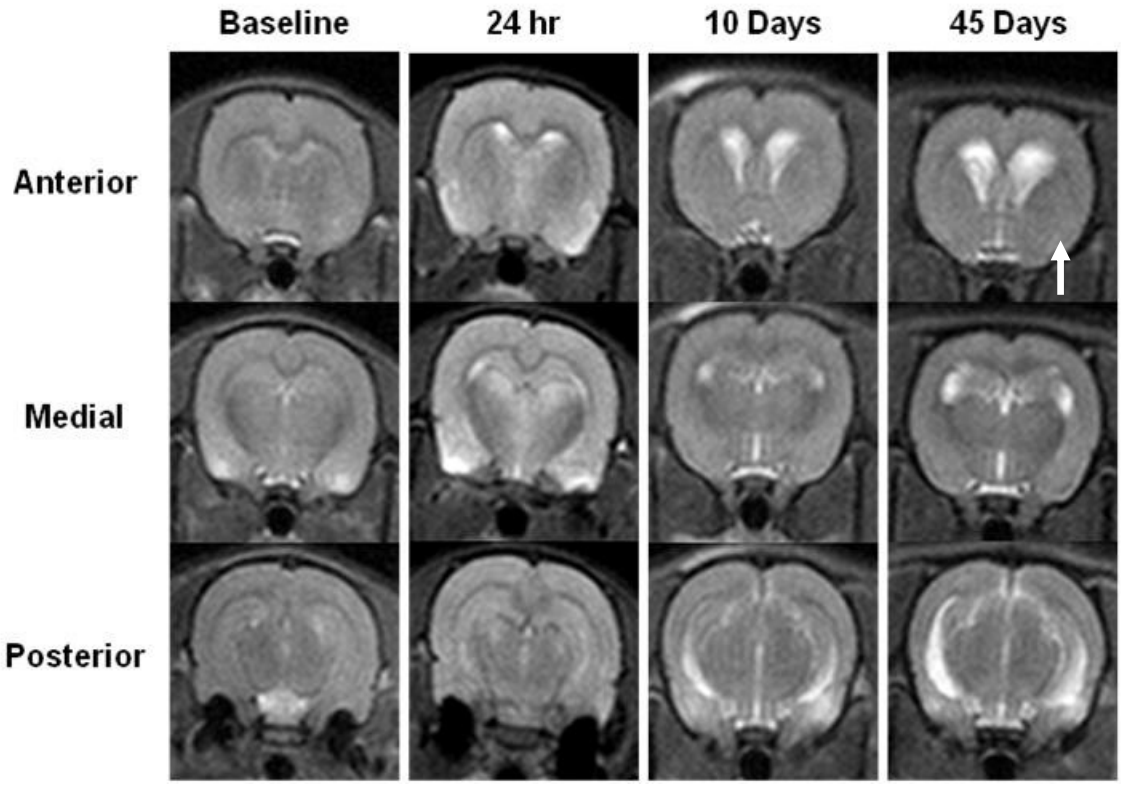


Figure 4.3. Representative brain MRI images taken using T2 weighted brain scans of four different rats at baseline and at 24 hrs, 10 and 45 days following 90 min of SE. Bright white areas indicate edema, particularly in the hippocampus (see arrow).

baseline (n=5) and at 24 hrs (n=5), 10 (n=3) and 45 (n=3) days following 90 min of SE. These are the same animals that underwent MRI analysis of the heart in Fig. 4.1. These images show considerable edema in the hippocampus and cortex in all animals that underwent SE procedures within 24 hrs, which persists through 10 days and becomes notably worse by 45 days. Additionally, these images also show an enlargement of the ventricles by day 45 following SE. Although cardiac function appears to recover within 10 days following SE, cerebral edema persists and does not recover by 45 days.

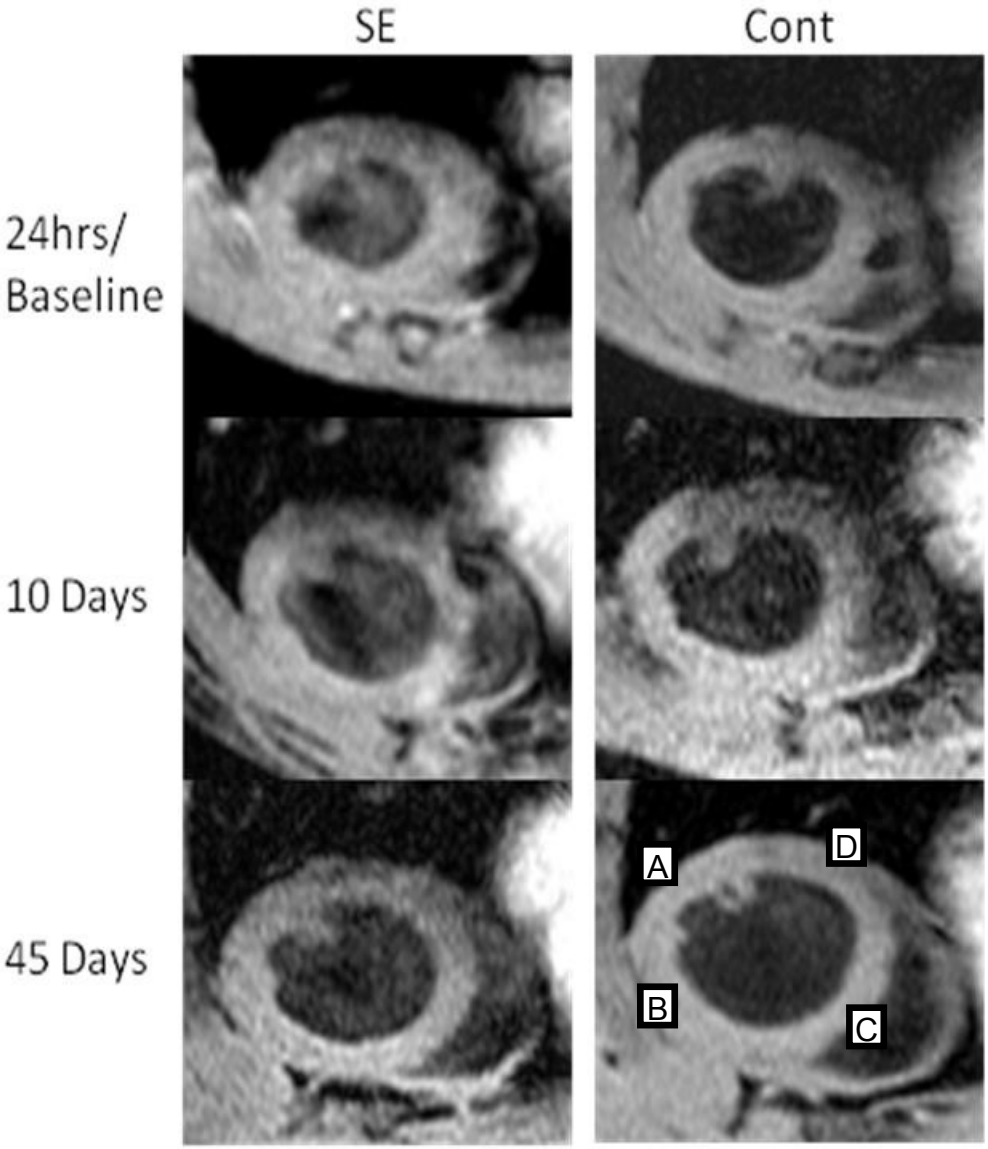
Cardiac Damage

Fig. 4.4 shows a representative image of a short axis view of the LV mid-cavity scans taken from both Cont and SE animals. All MRI scans taken from Cont (n=11) and SE (n=11) animals were visually evaluated for gross anatomical changes. No visible gross cardiac damage or infarctions were noted in any MRI image in any animal. These data support the proposal that SE produces only subtle and reversible cardiac damage that induces short-term contractile dysfunction—such as stunning and impaired lusitropy.

HR During MRI Scans

The average HR in Cont (24 hrs, n=5; 10 days, n=3; 45 days n=3) and SE (24 hrs, n=5; 10 days, n=3; 45 days n=3) animals was determined over 10-15 min periods while under anesthesia and are summarized in Fig. 4.5. SE

Figure 4.4. Representative images of cardiac mid-ventricular short axis slices during diastole at 24 hrs, 10 and 45 days following 90 min of SE. These images show increased wall thickness of the LV at 24hrs, but not at 10 or 45 days following SE. Further, no gross anatomical damage or remodeling of the LV was observed by 45 days. LV wall measurements were taken from inferolateral (A), anterolateral (B), anteriorseptal (C), inferospetal (D) regions.


SE
Cont
24hrs/
Baseline
10 Days
45 Days
A
B
C
D

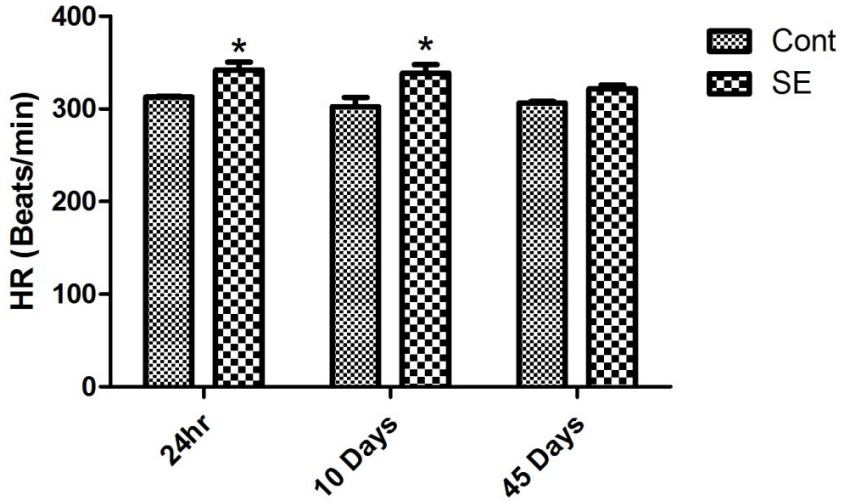


Figure 4.5. Average HR determined during MRI scans in Cont (24 hrs, n=5; 10 Days, n=3; 45 Days, n=3) and SE (24 hrs, n=5; 10 Days, n=3; 45 Days, n=3) animals while under isoflurane anesthetic (Cont, 1.5-2.0%; SE 2.5-3.0%). *= $p<0.05$ for SE vs. Cont.

animals demonstrated a significant increase in HR independent of respiration rate (maintained at 40-46 breaths/min) or oxygen saturation (maintained at >97%) while under anesthesia, and needed more isoflurane (Cont, 1.5-2.0%; SE 2.5-3.0%) to maintain the same plane of anesthesia.

Discussion

Results from these experiments demonstrate that within 24 hrs following seizure termination, SE induces reversible LV contractile dysfunctions that are similar to cardiac stunning in humans. SE produces both systolic and diastolic dysfunctions characterized by decreased LV stroke work, altered inotropy, impaired lusitropy, and increased LV wall thickness without irreversible gross cardiac damage. These data further demonstrate that these cardiac deficits recover within 1-10 days following SE cessation, as there were no apparent indications of altered contractility or gross remodeling of the LV beyond 10 days. Although cardiac recovery was demonstrated, cerebral edema in the hippocampus, cortex and ventricles persisted for more than 45 days, which suggests that CNS damage may alter the ANS and affect cardiac response. Together, these data support the proposal that SE induces a recoverable contractile dysfunction similar to Together, these data support the hypothesis that SE induces a recoverable contractile dysfunction similar to cardiac stunning in humans, which increases mortality risk. Although recovery from cardiac dysfunction may

reduce mortality risk factors, SE may produce more chronic alterations to autonomic balance, due to persistent CNS damage, which may prolong cardiac risk factors associated with sudden cardiac death.

Several studies in both humans and animals have demonstrated that SE produces intense activation of the sympathetic nervous system (Benowitz, et al. 1986), increased plasma catecholamine levels (Shimizu, et al. 2008, Walton 1993), tachycardia, and hypertension (Metcalf, et al. 2009a, Sakamoto, et al. 2008). In more recent studies, neurogenically mediated cardiac stunning has been reported in patients following an episode of SE (Legriel, et al. 2008, Shimizu, et al. 2008). These data support the proposal that an increased chronotropic and inotropic effect during SE produces tachycardiac ischemia and cardiac injury. Additionally, the hypertensive response to SE may also contribute to cardiac stunning due to increased afterload (Kloner and Jennings 2001). Together these studies suggest a mechanism by which SE produces neurogenically mediated cardiac stunning through intense activation of the SymNS and increased plasma catecholamine levels (Legriel, et al. 2008, Rona 1985, Simon, et al. 1984, Walton 1993, Wittstein, et al. 2005). It has been demonstrated in animals that Li-pilocarpine induced SE produces a chronotropic and inotropic response associated with cardiac injury (Bealer, et al. 2010, Metcalf, et al. 2009a); however, it was unknown if SE-induced a contractile dysfunction that was recoverable. The present experiments demonstrate that SE can produce

a recoverable contractile dysfunction similar to stunning in humans, demonstrated by decreased LV chamber volume, diminished lusitorpy, and increased ventricular wall thickness without gross cardiac damage—which returned to Cont values by 10 days.

Abnormalities of ventricular function can be separated into problems of ventricular filling (diastole) and ventricular empting (systole). Systolic ventricular dysfunction, as is precipitated by heart failure, refers to the loss of cardiac inotropy that results in a diminished stroke volume, a compensatory rise in end-diastolic pressure, and increased LV end-diastolic volume due to ventricular hypertrophy, dilation. Consequently, in heart failure, ejection fraction can become decreased as stroke volume decreases and end-diastolic volume increases. However, even in the presence of normal systolic ventricular performance (i.e., normal ejection fraction), abnormalities of diastolic ventricular filling can occur because of low ventricular compliance, thereby reducing stroke volume. Diastolic dysfunction in the setting of normal systolic function may be due to obstruction of left ventricular filling, impaired left ventricular distensibility, or active relaxation (Zile, et al. 2004). This results in appreciable reduction in both stroke volume and end-diastolic volume such that ejection fraction does not change. The present experiments demonstrate a reduction in both stroke volume and end-diastolic volume, which produced a slight increase in ejection fraction that is consistent with a diastolic dysfunction and not a systolic

dysfunction. It has been suggested that the mechanism of abnormal ventricular relaxation is due to impaired excitation-contraction coupling and Ca^{2+} regulation (see Chapter 1).

Chronic effects and mechanisms of recovery following myocardial stunning are unclear. Previous research has shown that, in animals, SE produces chronic alterations including LV ion channel remodeling (Bealer, et al. 2010), increased SymNS tone (Metcalf, et al. 2009b), arrhythmogenic changes to ECG and increased susceptibility to arrhythmias (Metcalf, et al. 2009a) within 10 days. Experiments presented here demonstrate a cardiac dysfunction that recovers within 10 days, despite an elevated resting HR in anesthetized animals that have undergone SE. We proposed that cardiac injury produced by SE may act as a substrate for adaptive compensatory cardiac remodeling to occur. Thus, increased HR is likely due to altered ANS balance in response to CNS damage and is a potential compensatory mechanism to diminished cardiac efficiency. Indeed it remains unknown, but is likely that cardiac recovery depends on adaptive compensatory cardiac remodeling within 10 days following SE cessation. Consequently, these cardiac alterations may increase risk factors that predispose patients to sudden cardiac death beyond the stunning period.

In contrast to the transient LV dysfunction which appears to recover within 10 days following SE, cerebral edema persists and even worsens by 45 days. The effects of seizure activity on brain damage and the association to

cardiac pathology are not clear. However, it has been shown that SE does produce chronic alterations in SymNS balance in the weeks following SE (Metcalf, et al. 2009b). Additionally, seizures, edema, or damage that affects areas in the central autonomic network may alter autonomic afferents or modify autonomic expression (Devinsky 2004). More specifically, these autonomic networks encompass cortical limbic areas, including the amygdala and insular cortex, which directly connects with subcortical regions of the central autonomic network, including the hypothalamus, the pons, solitary tract nucleus, and ventrolateral medulla (Devinsky 2004, Kanter, et al. 1991, Kanter, et al. 1996). The T2-weighted images presented in Fig. 4.2 demonstrate pervasive edema through the limbic and cortical systems. Since these brain regions are critical in regulating SymNS activity, excitatory stimulation, damage or alteration to these brain areas could result in altered SymNS tone and increased susceptibility to sudden cardiac death.

Isoflurane is known to increase heart rate and decrease respiration rate in a dose-dependent manner and greatly depends upon body temperature and oxygen saturation (Pacher, et al. 2008). The reason for the increased dose of isoflurane necessary to reach the same plane of anesthesia following SE is not clear; however, it is likely that central nervous system damage or increased SymNS tone could alter the pharmacodynamics and pharmacokinetics of isoflurane. Alternatively, increased HR also may be a compensatory response to decreased cardiac function or stress caused by

SE—further investigation is needed to clarify differential affects of anesthesia following SE.

Animals that underwent Li-pilocarpine -SE can develop spontaneous seizures in the months following recovery. Although these animals were not video monitored, no observable motor seizures were noted from the time of SE termination with VPA through 45 days.

Following an episode of SE patients are at an increased risk for mortality by an unknown mechanism. Cardiac stunning has been reported in patients following prolonged seizure activity, which increases mortality risk. The present experiments demonstrate that SE can produce a reversible contractile dysfunction in rodents similar to cardiac stunning humans. Further, these data demonstrate that cardiac dysfunction resulting from SE can recover independently of brain damage. However, cerebral edema may contribute to alterations in sympathovagal balance. Together these results suggest prolonged seizures cause neurogenically mediated cardiac stunning, which increase mortality risk. Cardiac stunning is a potentially preventable and treatable disorder that warrants cardiac monitoring of all patients following severe and prolonged seizure activity.

References

Bealer SL, Little JG, Metcalf CS, Brewster AL, Anderson AE. (2010) Autonomic and cellular mechanisms mediating detrimental cardiac effects of status epilepticus. *Epilepsy Res* 91:66-73.

Benowitz NL, Simon RP, Copeland JR. (1986) Status epilepticus: divergence of sympathetic activity and cardiovascular response. *Ann Neurol* 19:197-199.

Bolli R, Marban E. (1999) Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* 79:609-634.

Devinsky O. (2004) Effects of Seizures on Autonomic and Cardiovascular Function. *Epilepsy Curr* 4:43-46.

Glien M, Brandt C, Potschka H, Voigt H, Ebert U, Loscher W. (2001) Repeated low-dose treatment of rats with pilocarpine: low mortality but high proportion of rats developing epilepsy. *Epilepsy Res* 46:111-119.

Jain R, Deveikis J, Thompson BG. (2004) Management of patients with stunned myocardium associated with subarachnoid hemorrhage. *AJNR Am J Neuroradiol* 25:126-129.

Kanter RK, Erickson JT, Millhorn DE. (1991) Activation of the c-fos gene in prodynorphin- and proenkephalin-expressing cells of the nucleus tractus solitarius after seizures. *Exp. Neurol.* 129:290-298.

Kanter RK, Strauss JA, Sauro MD. (1996) Comparison of neurons in rat medulla oblongata with fos immunoreactivity evoked by seizures, chemoreceptor, or baroreceptor stimulation. *Neurosci.* 73:807-816.

Kloner RA, Jennings DB. (2001) Consequences of breif ischemia: stunning, predonditioning, and their clinical implications. *Circulation* 104:3158-3167.

Kulkarni SK, George B. (1995) Lithium-pilocarpine neurotoxicity: a potential model of status epilepticus. *Methods Find. Exp. Clin. Pharamacol.* 17:551-567.

Legriel S, Bruneel F, Dalle L, Appere-de-Vecchi C, Georges JL, Abbosh N, Henry-Lagarrigue M, Revault D'Allonnes L, Ben Mokhtar H, Audibert J, Guezennec P, Troche G, Bedos JP. (2008) Recurrent takotsubo cardiomyopathy triggered by convulsive status epilepticus. *Neurocrit Care* 9:118-121.

Metcalf CS, Poelzing S, Little JG, Bealer SL. (2009a) Status epilepticus induces cardiac myofilament damage and increased susceptibility to arrhythmias in rats. *Am J Physiol Heart Circ Physiol* 297:H2120-2127.

Metcalf CS, Radwanski PB, Bealer SL. (2009b) Status epilepticus produces chronic alterations in cardiac sympathovagal balance. *Epilepsia* 50:747-754.

Pacher P, Nagayama T, Mukhopadhyay P, Batkai S, Kass DA. (2008) Measurement of cardiac function using pressure-volume conductance catheter technique in mice and rats. *Nat Protoc* 3:1422-1434.

Pomblum VJ, Korbmacher B, Cleveland S, Sunderdiek U, Klocke RC, Schipke JD. (2010) Cardiac stunning in the clinic: the full picture. *Interact CardioVasc Thorac Surg* 10:86-91.

Racine RJ. (1972) Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 32:281-294.

Rona G. (1985) Catecholamine cardiotoxicity. *J Mol Cell Cardiol* 17:291-306.

Sakamoto K, Saito T, Orman R, Koizumi K, Lazar J, Salciccioli L, Stewart M. (2008) Autonomic consequences of kainic acid-induced limbic cortical seizures in rats: Peripheral autonomic nerve activity, acute cardiovascular changes, and death. *Epilepsia*:1-15.

Shimizu M, Kagawa A, Takano T, Masai H, Miwa Y. (2008) Neurogenic stunned myocardium associated with status epilepticus and postictal catecholamine surge. *Intern. Med.* 47:269-273.

Simon RP, Aminoff MJ, Benowitz NL. (1984) Changes in plasma catecholamines after tonic-clonic seizures. *Neurology* 34:255-257.

Walton NY. (1993) Systemic effects of generalized convulsive status epilepticus. *Epilepsia* 34 Suppl 1:S54-58.

Wittstein IS, Thiemann DR, Lima JA, Baughman KL, Schulman SP, Gerstenblith G, Wu KC, Rade JJ, Bivalacqua TJ, Champion HC. (2005) Neurohumoral features of myocardial stunning due to sudden emotional stress. *N Engl J Med* 352:539-548.

Zile MR, Baicu CF, Gaasch WH. (2004) Diastolic heart failure--abnormalities in active relaxation and passive stiffness of the left ventricle. *N Engl J Med* 350:1953-1959.

CHAPTER 5

EX VIVO MEASUREMENTS OF CALCIUM TRANSIENTS FOLLOWING STATUS EPILEPTICUS

Introduction

Intracellular calcium (Ca^{2+}) concentration in myocytes is one of the most important factors contributing to increased myocardial contraction and relaxation. It has been proposed that cardiac stunning alters excitation-contraction band coupling and represents a favorable substrate for triggered arrhythmias due to enhanced susceptibility to Ca^{2+} overload (Gao, et al. 1995) and Ca^{2+} myofilament proteolysis (Gao, et al. 1997). Previously (see Chapter 4) MRI scans demonstrated a reversible cardiac dysfunction, similar to stunning, characterized by incomplete relaxation of the left ventricle following status epilepticus (SE). The mechanism that impairs myocardial relaxation is believed to involve alterations in Ca^{2+} handling (Apstein and Lorell 1988). Thus, we propose that status epilepticus alters cardiac function through changes in Ca^{2+} regulation and sensitivity to cardiac troponin. To determine if Ca^{2+} regulation is altered following SE, *ex vivo* experiments

were preformed to measure the time constant of cytosolic Ca^{2+} decline (tau) using isolated hearts perfused with the Ca^{2+} indicator Indo-1-AM.

Methods

Animals

Retired breeder Hartley Guinea pigs were obtained from a commercial supplier (Charles River, Wilmington, MA). Animals were individually housed with *ad libitum* access to food and water and maintained at 22°C on a 12 hr: 12 hr, light: dark schedule. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Utah.

Status Epilepticus

SE was induced using lithium-pilocarpine, using previously described methods (Uva, et al. 2008). Briefly, an injection of lithium (127 mM/kg ip, Sigma) was given 18-24 hrs prior to pretreatment with methylscopalamine (1 mg/kg ip, 30 min, Sigma) and administration of pilocarpine (100 mg/kg ip, Sigma). Control animals were administered saline vehicle in place of pilocarpine. The modified Racine Scale was used to evaluate seizure activity (Racine 1972). Approximately 30 min following pilocarpine treatment stage 4-5 seizures were observed. After 90 min of SE, seizure activity was terminated with valproic acid (400 mg/kg ip, Sigma).

Guinea Pig Langendorff Heart Preparation

Guinea pig ventricles were perfused as Langendorff preparations which is described elsewhere (Poelzing and Veeraraghavan 2007, Radwanski, et al. 2010). Briefly, guinea pigs (0.8-1.0 kg) were anesthetized with sodium pentobarbital (30 mg/kg ip). Next, their hearts were rapidly excised, the atria removed and perfused as Langendorff preparations (perfusion pressure 55 mmHg) with oxygenated (100% O_2) Tyrode solution at 36.5°C of the following composition (in mM): $CaCl_2$ 2, NaCl 140, KCl 4.5, dextrose 10, $MgCl_2$ 1, and HEPES 10 (pH 7.41).

Optical Mapping-Calcium

A calcium mapping system, developed by the Poelzing laboratory at the University of Utah, was used in these experiments as previously described (Radwanski, et al. 2010). Briefly, cardiomyocyte Ca^{2+} handling dynamics were quantified by optical mapping of intracellular Ca^{2+} with the calcium indicator Indo-1-AM. Intracellular Ca^{2+} was measured using dual wavelength collection. Excitation light obtained from a 1000-W mercury arc lamp was filtered at 350 ± 10 nm and directed through a flexible liquid light guide to the preparation. Fluorescent light from the preparation was collected by a 150 mm achromatic lens (BK7/Flint, Ealing, Rocklin, CA) incident on a 515 DCXR dichroic mirror set at a 45° angle to the recording surface. Fluoresced light due to the Ca^{2+} was reflected off the 515 DCXR dichroic

mirror onto a 445-nm dichroic long-pass mirror which was positioned between the lenses of the tandem lens assembly at a 45° angle. All wavelengths above 445 nm were transmitted to one CCD and wavelengths below 445 nm to another CCD camera. For ratiometric optical mapping as described previously (Laurita, et al. 2003), transmitted and reflected fluorescent light was limited to 485 ± 10 nm and 405 ± 10 nm, respectively, by using optical interference filters. The ratiometric calcium transients (arbitrary units (AU)) were calculated as follows (Katra, et al. 2004): $[Ca^{2+}]_i = (CaF_{405}\text{-}BF_{405})/(CaF_{485}\text{-}BF_{485})$, where CaF_{405} and CaF_{480} were the signal intensities during Indo-1 perfusion in the 405 nm and 480 nm filtered signals. BF_{405} and BF_{485} were the signal intensities from the 405 and 480 nm filtered signals in the absence of Indo-1. This ratio has been previously used to demonstrate differences in diastolic Ca^{2+} concentration and Ca^{2+} transient amplitude between experiments (Katra, et al. 2004).

Optical Measurements

Motion was further reduced by perfusion with 1.5 mM 2,3-butanedione monoxime (BDM). Hearts were stimulated with a unipolar silver wire placed on the anterior epicardial surface close to the equatorial plane of the ventricle being mapped at 1.5 times the stimulation threshold with a basic cycle length (BCL) of 200ms. To quantify the rate of intracellular Ca2+ recovery to diastolic levels, the decay portion of the Ca^{2+} transient (from 70% to 0% of the

decline phase) was measured as the time constant (τ) of a single exponential fit as previously described (Bers and Berlin 1995, Clark, et al. 1996, Katra, et al. 2004).

Arrhythmia Induction

Procedures for rapid pacing induced arrhythmias are described in detail elsewhere (Radwanski, et al. 2010). Briefly, a 20-beat drive train stimulus (S1; at BCL of 400 ms) was delivered to the anterior epicardial surface of the right ventricular base. Using the same drive train a second premature stimulus (S2) was delivered to the epicardial left ventricular apex. To reach refractoriness or induce arrhythmias, the S1-S2 interval was sequentially shortened by 10 ms. Susceptibility to rapid pacing–induced arrhythmias was determined using the shortest cycle length for S1-S2 interval that maintained 1:1 capture. A premature ventricular complex (PVC) was defined as any nonrecurring QRS complex that occurred with in <1.5 SD of the intrinsic cycle length (Radwanski, et al. 2010).Ventricular tachycardia (VT) was defined as a series of ≥ three premature ventricular beats.

Results

Pacing -induced Arrhythmias

Following 90 min of pilocarpine induced SE, three of four animals had VT induced with one animal losing 1:1 capture before arrhythmias could be

induced. Twenty-four hours following SE, one of three animals had VT induced with two animals losing 1:1 capture before arrhythmias could be induced. These results suggest that increased susceptibility to arrhythmias acutely following 90 min of SE, but not at 24 hrs when compared to Cont.

Ex Vivo Calcium Transients

Fig. 5.1 shows rate of Ca^{2+} transient, tau, at BCL 200 ms measured in Cont (n=4), 90 min following SE (n=3), and 24 hrs following SE (n=3). These data show no significant difference in the Ca^{2+} transients following SE at either time point compared to the Cont.

Discussion

These studies demonstrate that at either 0 hrs or 24 hrs following 90 min of SE, Ca^{2+} transients were not altered in the Langendorff isolated heart preparation. These results suggest that the mechanism of arrhythmias may not be mediated by Ca^{2+} dysregulation intrinsic to the heart. Further, since these hearts are denervated from autonomic influence, these results suggest that following prolonged seizure activity an intact autonomic nervous system is necessary to constitute cardiac dysfunction and manifest both acute and chronic adaptations.

Previously it has been reported in patients and animal models that SE induces a reversible decrement in cardiac function (Aminoff and Simon 1980,

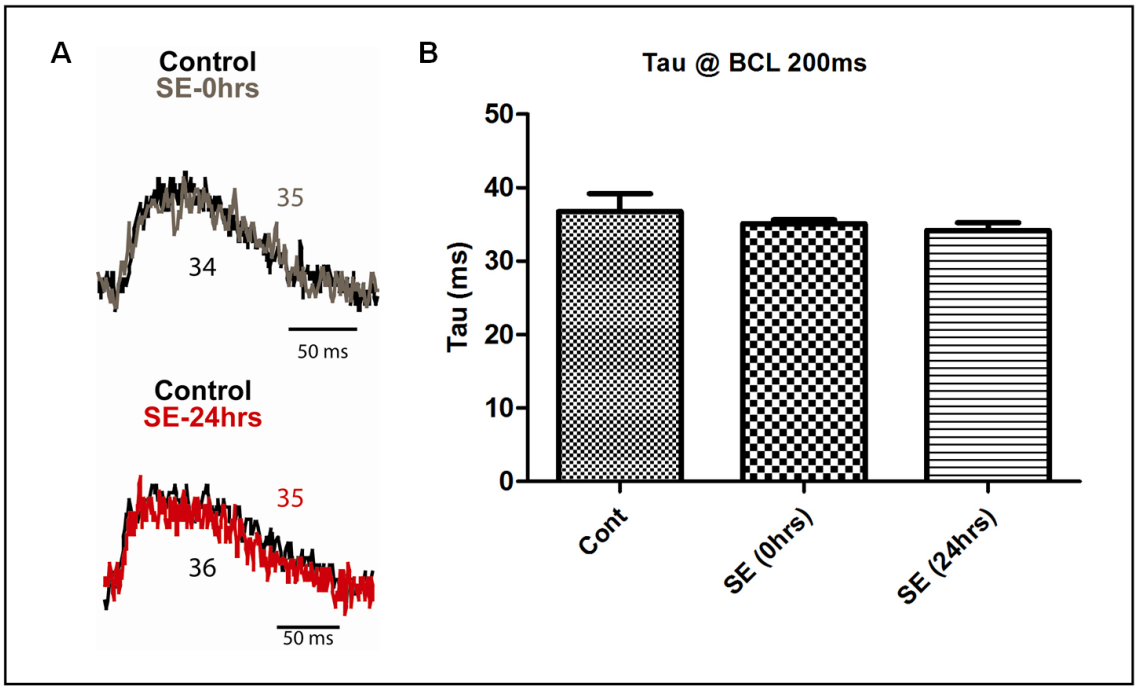


Figure 5.1. Panel A shows *Ex Vivo* Ca^{2+} transients at 0 hrs (n=3) and 24 hrs (n=3) following SE and in cont (n=3) guinea pigs. Panel B shows tau, a measurement of Ca^{2+} decay, at a basic cycle length (BCL) of 200 ms. No significant difference in Ca^{2+} transients was observed in any SE animal when compared with control.

Kreisman, et al. 1993, Legriel, et al. 2008, Manno, et al. 2005, Painter, et al. 1993, Sakuragi, et al. 2007, Young, et al. 1985), similar to cardiac stunning (Pomblum, et al. 2010), which is characterized by a myofilament loss of cardiac troponin, increased sympathetic tone, altered cardiac hemodynamic, prolonged QT interval, and increased susceptibility to arrhythmias (Bealer, et al. 2010, Metcalf, et al. 2009a, Stollberger and Finsterer 2004). While all these changes are known cardiac risk factors that increase mortality, the mechanism(s) of cardiac stunning and increased susceptibility to arrhythmias following SE remains unknown.

One potential mechanism that may produce both stunning and arrhythmias following prolonged seizure activity is intense sympathetic activation and catecholamine mediated tachycardic ischemia. The major mechanism of intracellular diastolic Ca^{2+} regulation is discussed in detail in Chapter 1. Generally, prolonged tachycardia can induce intracellular diastolic Ca^{2+} overload that damages and impairs myofilament function (Carroll, et al. 1983). As a result, cardiac electrophysiology is altered producing a contractile dysfunction that increases arrhythmogenic activity (Anderson 2003, Bernstein, et al. 2000, Devinsky 2004, Freeman 2006, Lathers and Schraeder 1987, Metcalf, et al. 2009b, Nei, et al. 2000, Opherk, et al. 2002, Ryvlin, et al. 2006, Teplitz, et al. 2005). Consequently, intracellular Ca^{2+} dysregulation that results from prolonged sympathetic

activation may result in diastolic dysfunction, such as cardiac stunning (Gao, et al. 1995, Przyklenk, et al. 1987).

A major hallmark of a diastolic dysfunction induced by ischemic injury is impaired myocardial relaxation (Gilbert and Glantz 1989, Lorell 1991). Various studies have shown that there is a relationship between the time constant of left ventricular pressure decline, a measure of impaired relaxation, and tau, the time constant of cytosolic Ca^{2+} decline (Applegate, et al. 1987, Serizawa, et al. 1987, Serizawa, et al. 1981). Thus, the mechanisms that produce impaired myocardial relaxation following ischemic injury are believed to involve abnormalities of Ca^{2+} handling. As discussed in previous Chapters, both MRI scans and cardiac output measurements in rats following SE demonstrate impaired myocardial relaxation following seizure cessation; however, results of these experiment demonstrate that Tau is not altered in the isolated guinea pig heart and suggest Ca^{2+} dysregulation, due to altered cytosolic reuptake, does not mediate cardiac diastolic dysfunction following SE. This would indicate that diastolic dysfunction is not intrinsic to the heart and may depend on external factors such as autonomic tone, circulating catecholamines, afferent information to higher cardiac brain centers, endocrine effects, and hemodynamic factors.

Several limitations of these experiments should be considered in the interpretation of these data and when considering potential mechanisms of arrhythmias and cardiac dysfunction following SE. First, increases in Ca^{2+}

transients may be too small to be detected by this method; even subtle changes in Ca^{2+} regulation could potentially increase susceptibility to arrhythmias and alter cardiac function. Second, the perfusion time during the Langendorff preparation may act to rescue cardiac myocytes from tachycardic ischemia by removing sympathetic influence and adrenoceptor mediated regulation. Third, cardiac arrhythmias produced by atrial fibrillation or damage to pace maker cells may be prevented by removal of the atria as part of the Langedorff preparation. SE-induced changes in automaticity are unknown. Fourth, a longer period of recovery following SE may be necessary to manifest adaptive cardiac remodeling. Remodeling of potassium channels LV myocytes has been shown to occur within 10 ten days of SE (Bealer, et al. 2010); however, changes in ion channel expression before this time point are unknown.

Results of these experiments suggest that Ca^{2+} transients, under the conditions studied and given the four caveats listed above, are not major contributors to diastolic dysfunction in the isolated guinea pig heart following an episode of SE. However, results of these studies may be inconclusive due to the absence of SymNS influence and a lack of sensitivity likely necessary to detect a more subtle effect. Moreover, these results are in contradiction with diastolic dysfunctions observed in rats (see Chapter 4).

References

Aminoff MJ, Simon RP. (1980) Status epilepticus. Causes, clinical features and consequences in 98 patients. *Am J Med* 69:657-666.

Anderson KP. (2003) Sympathetic nervous system activity and ventricular tachyarrhythmias: recent advances. *Ann Noninvasive Electrocardiol* 8:75-89.

Applegate RJ, Walsh RA, O'Rourke RA. (1987) Effects of nifedipine on diastolic function during brief periods of flow-limiting ischemia in the conscious dog. *Circulation* 76:1409-1421.

Apstein CS, Lorell BH. (1988) The physiological basis of left ventricular diastolic dysfunction. *J Card Surg* 3:475-485.

Bealer SL, Little JG, Metcalf CS, Brewster AL, Anderson AE. (2010) Autonomic and cellular mechanisms mediating detrimental cardiac effects of status epilepticus. *Epilepsy Res* 91:66-73.

Bernstein R, Mayer SA, Magnano A. (2000) Neurogenic stunned myocardium in Guillain-Barre syndrome. *Neurology* 54:759-762.

Bers DM, Berlin JR. (1995) Kinetics of [Ca]i decline in cardiac myocytes depend on peak [Ca]i. *Am J Physiol* 268:C271-277.

Carroll JD, Hess OM, Hirzel HO, Krayenbuehl HP. (1983) Exercise-induced ischemia: the influence of altered relaxation on early diastolic pressures. *Circulation* 67:521-528.

Clark RB, Bouchard RA, Giles WR. (1996) Action potential duration modulates calcium influx, Na(+)-Ca2+ exchange, and intracellular calcium release in rat ventricular myocytes. *Ann N Y Acad Sci* 779:417-429.

Devinsky O. (2004) Effects of Seizures on Autonomic and Cardiovascular Function. *Epilepsy Curr* 4:43-46.

Freeman R. (2006) Assessment of cardiovascular autonomic function. *Clin Neurophysiol* 117:716-730.

Gao WD, Atar D, Backx PH, Marban E. (1995) Relationship between intracellular calcium and contractile force in stunned myocardium. Direct evidence for decreased myofilament Ca2+ responsiveness and altered diastolic function in intact ventricular muscle. *Circ Res* 76:1036-1048.

Gao WD, Atar D, Liu Y, Perez NG, Murphy AM, Marban E. (1997) Role of troponin I proteolysis in the pathogenesis of stunned myocardium. *Circ Res* 80:393-399.

Gilbert JC, Glantz SA. (1989) Determinants of left ventricular filling and of the diastolic pressure-volume relation. *Circ Res* 64:827-852.

Katra RP, Pruvot E, Laurita KR. (2004) Intracellular calcium handling heterogeneities in intact guinea pig hearts. *Am J Physiol Heart Circ Physiol* 286:H648-656.

Kreisman NR, Gauthier-Lewis ML, Conklin SG, Voss NF, Barbee RW. (1993) Cardiac output and regional hemodynamics during recurrent seizures in rats. *Brain Res* 626:295-302.

Lathers CM, Schraeder PL. (1987) Review of autonomic dysfunction, cardiac arrhythmias, and epileptogenic activity. *J Clin Pharmacol* 27:346-356.

Laurita KR, Katra R, Wible B, Wan X, Koo MH. (2003) Transmural heterogeneity of calcium handling in canine. *Circ Res* 92:668-675.

Legriel S, Bruneel F, Dalle L, Appere-de-Vecchi C, Georges JL, Abbosh N, Henry-Lagarrigue M, Revault D'Allonnes L, Ben Mokhtar H, Audibert J, Guezennec P, Troche G, Bedos JP. (2008) Recurrent takotsubo cardiomyopathy triggered by convulsive status epilepticus. *Neurocrit Care* 9:118-121.

Lorell BH. (1991) Significance of diastolic dysfunction of the heart. *Annu Rev Med* 42:411-436.

Manno EM, Pfeifer EA, Cascino GD, Noe KH, Wijdicks EF. (2005) Cardiac pathology in status epilepticus. *Ann Neurol* 58:954-957.

Metcalf CS, Poelzing S, Little JG, Bealer SL. (2009a) Status epilepticus induces cardiac myofilament damage and increased susceptibility to arrhythmias in rats. *Am J Physiol Heart Circ Physiol* 297:H2120-2127.

Metcalf CS, Radwanski PB, Bealer SL. (2009b) Status epilepticus produces chronic alterations in cardiac sympathovagal balance. *Epilepsia* 50:747-754.

Nei M, Ho RT, Sperling MR. (2000) EKG abnormalities during partial seizures in refractory epilepsy. *Epilepsia* 41:542-548.

Opherk C, Coromilas J, Hirsch LJ. (2002) Heart rate and EKG changes in 102 seizures: analysis of influencing factors. *Epilepsy Res* 52:117-127.

Painter JA, Shiel FO, DeLorenzo RJ. (1993) Cardiac pathology findings in status epilepticus. *Epilepsia* 34 Suppl 6:30.

Poelzing S, Veeraraghavan R. (2007) Heterogeneous ventricular chamber response to hypokalemia and inward rectifier potassium channel blockade underlies bifurcated T wave in guinea pig. *Am J Physiol Heart Circ Physiol* 292:H3043-3051.

Pomblum VJ, Korbmacher B, Cleveland S, Sunderdiek U, Klocke RC, Schipke JD. (2010) Cardiac stunning in the clinic: the full picture. *Interact CardioVasc Thorac Surg* 10:86-91.

Przyklenk K, Patel B, Kloner RA. (1987) Diastolic abnormalities of postischemic "stunned" myocardium. *Am J Cardiol* 60:1211-1213.

Racine RJ. (1972) Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 32:281-294.

Radwanski PB, Veeraraghavan R, Poelzing S. (2010) Cytosolic calcium accumulation and delayed repolarization associated with ventricular arrhythmias in a guinea pig model of Andersen-Tawil syndrome. *Heart Rhythm* 7:1428-1435 e1421.

Ryvlin P, Montavont A, Kahane P. (2006) Sudden unexpected death in epilepsy: from mechanisms to prevention. *Curr Opin Neurol* 19:194-199.

Sakuragi S, Tokunaga N, Okawa K, Kakishita M, Ohe T. (2007) A case of takotsubo cardiomyopathy associated with epileptic seizure: reversible left ventricular wall motion abnormality and ST-segment elevation. *Heart Vessels* 22:59-63.

Serizawa T, Momomura S, Kohmoto O, Ohya T, Sato H, Takahashi T, Mochizuki T, Iizuka M, Sugimoto T. (1987) Mechanisms of abnormal myocardial relaxation induced by ischemia: comparison of low flow ischemia and hypoxia in isolated rabbit heart. *Jpn Circ J* 51:90-97.

Serizawa T, Vogel WM, Apstein CS, Grossman W. (1981) Comparison of acute alterations in left ventricular relaxation and diastolic chamber stiffness induced by hypoxia and ischemia. Role of myocardial oxygen supply-demand imbalance. *J Clin Invest* 68:91-102.

Stollberger C, Finsterer J. (2004) Cardiac troponin levels following monitored epileptic seizures. *Neurology* 62:1453.

Teplitz L, Igic R, Berbaum ML, Schwertz DW. (2005) Sex differences in susceptibility to epinephrine-induced arrhythmias. *J Cardiovasc Pharmacol* 46:548-555.

Uva L, Librizzi L, Marchi N, Noe F, Bongiovanni R, Vezzani A, Janigro D, de Curtis M. (2008) Acute induction of epileptiform discharges by pilocarpine in the in vitro isolated guinea-pig brain requires enhancement of blood-brain barrier permeability. *Neuroscience* 151:303-312.

Young RS, Fripp RR, Yagel SK, Werner JC, McGrath G, Schuler HG. (1985) Cardiac dysfunction during status epilepticus in the neonatal pig. *Ann Neurol* 18:291-297.

CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

Summary

During the 30-day period following status epilepticus (SE), patients are at an increased risk for mortality. Previously, it has been demonstrated that SE produces a sudden and prolonged activation of the sympathetic nervous system (Benowitz, et al. 1986) and hypersecretion of catecholamines that act to overstimulate ß-adrenergic receptors on cardiac muscle (Walton 1993).

Using these prior observations, we proposed that cardiac overstimulation produces tachycardic ischemia reperfusion and contractile dysfunction following SE termination—which are well established risk factors for sudden cardiac death. Further, we proposed that a sympathetic blockade would prevent SE-induced cardiac myofilament damage, thereby reducing mortality risk.

To test these hypotheses, several different animal models of SE were used, with and without administration of a ß-1 receptor antagonist, to evaluate cardiac hemodynamic parameters, left ventricular function, cardiac damage, myocardial remodeling, cardiac electrical activity, and chemically

induced susceptibility to arrhythmias at various time points following seizure cessation.

Chapter 2 of this dissertation investigated the effect of intense activation of the SymNS on BP, HR, cardiac damage, alterations in cardiac electrical activity, susceptibility to arrhythmias and potential cardiac protection with a ß-1 receptor antagonist using a rat model of pilocarpine induced SE. Results from these studies demonstrated that SE produced hypertension, tachycardia, cardiac damage, and increased susceptibility to arrhythmias. Further, these results also demonstrated that cardiac therapy during prolonged seizure activity with a ß-1 adrenergic antagonist significantly prevented sympathetically mediated tachycardia, cardiac damage, arrhythmogenic electrical alterations, and reduced susceptibility to arrhythmias for 10-12 days following SE termination. However, administration of a ß -1 receptor antagonist did not prevent hypertension during SE. These findings demonstrated that cardiac effect of SE are potentially preventable with appropriate cardiac therapy and thereby reduce SE related mortality.

Although a loss of cTnI was demonstrated to occur during SE, which indicates impaired excitation-contraction machinery and suggests impaired inotropy, it does not completely explain cardiac stunning following seizure cessation. Several studies have shown that stunned myocardium responds well to inotropic agents (Bolli and Marban 1999, Choi, et al. 2010, Gao, et al.

1995), indicating contractile reserves remains and suggest that cardiac troponins may not be damaged sufficiently to independently limit inotropy. It is not yet clear what else may contribute to cardiac stunning such as, damaged or inactivated membrane bound receptors like ß-1 adrenergic receptor, ion channels, ryanodine receptors, or phospholamban. Alternatively, remodeling of SymNS innervations may also reflect changes in inotropy and lusitropy. In any case, the unique contributions to cardiac stunning by different myocyte components remain to be investigated.

Chapter 3 of this dissertation further investigated potential effects of ß -1 receptor overstimulation during SE and effects of adrenergic antagonism in a rat model of unilaterally stimulated SSLSE. More specifically, we investigated the proposal that SE results in diminished cardiac function through a neurogenic mechanism, such as cardiac stunning, in which increased sympathetic tone and high levels of plasma catecholamines induce cardiac overstimulation and produce cardiac dysfunction. Results of these studies demonstrate diminished cardiac ventricular function within 24 hrs, characterized by reduced CO, LVP dP/dt Max and Min, which is consistent with neurogenically mediated cardiac stunning in humans. Further, these studies show that administration of the ß-1 receptor antagonist during SSLSE prevents development of cardiac contractile dysfunctions, which occur within 24 hrs. Moreover, SSLSE produced a cardiac dysfunction that was similarly observed in the Li-pilocarpine model of SE in rats and demonstrates

that it is SE and not the muscarinic effects of pilocarpine that are mediating cardiac dysfunction. Together these results provide a potential mechanism and therapeutic strategy to reduce mortality risk by prevention of adverse cardiac effects caused by SE.

In Chapter 4 MRI scans were used to evaluate LV function acutely and chronically following SE using a rat model of pilocarpine induced SE. Results from these studies demonstrate that SE induces, albeit recoverable, LV contractile dysfunctions similar to cardiac stunning in humans. SE produces a diastolic dysfunctions characterized by decreased LV stroke volume; end-diastolic volume; near normal ejection fraction, indicative of impaired lusitropy; and increased LV wall thickness, without irreversible gross damage or apparent remodeling. MRI scans of the brain, however, did demonstrate gross damage and edema throughout the hippocampus, cortex, and ventricles, which persisted for more than 45 days.

Results from nearly every experiment indicated a diastolic dysfunction, including: prolonged QT interval at 24 hrs and 2 weeks, with no appreciable change in QRS; pressure measurements demonstrated a reduction in LVP dP/dt min, indicative of reduced relaxation; MRI imaging confirmed decreased SV and end-diastolic volume, with near normal ejection fraction. The primary mechanism whereby reduced lusitropy occurs is most likely near the end of the cycle of excitation-contraction coupling in the myocyte. At this point, the sarcoplasmic reticulum actively sequesters Ca^{2+} so that the

concentration of Ca^{2+} in the vicinity of the sacromere is rapidly reduced, allowing the Ca^{2+} to leave its binding sites on troponin-C and mediates actin disengagement from myosin. This step allows for rapid and complete relaxation of the myocyte. If SE induces or impairs the reduce rate of Ca^{2+} sequestration, by oxidative damage of SERCA or activation of calpains that degrade TnI for example, then the rate and extent of relaxation can become decreased. Ultimately, this will reduce the rate of ventricular filling resulting in a diastolic dysfunction.

In Chapter 5, we investigated Ca^{2+} dysregulation as potential mechanism that produces SE-induced cardiac dysfunction using a guinea pig model of pilocarpine induced SE. Results of these studies suggest that altered Ca^{2+} transients are not a mechanism of SE-induced cardiac stunning. However, these results are not consistent with the *in vivo* data collected in Chapters 2, 3, and 4, which suggests a diastolic dysfunction. Further, these experiments have several limitations that are discussed in Chapter 5. Notably, isolated cardiac preparations are essentially denervated and lack autonomic influence and afferent feedback mechanism to higher brain centers that affect cardiac regulation. This is highly suggestive that an intact autonomic nervous system or cardiac brain centers contributes directly to altered cardiac function.

While these studies have primarily focused on cardiac effects of SE, similar mechanisms likely occur with closely repeated seizures, which may

contribute to cardiac risk factors associated with sudden unexplained death in epilepsy (SUDEP). Seizures have been shown to exert effects on cardiac function (Schuele 2009) and are predominantly associated with tachyarrhythmias, and less frequently with bradyarrhythmias (Kerling, et al. 2009). Kerling, et al. demonstrated that cardiac postganglionic denervation occurred in patients with epilepsy and may be responsible for ictal asystole. These investigators conclude that altered cardiac SymNS innervations restrict heart rate modulation and indicate a postganglionic cardiac catecholamine disturbance, which increases risk of arrhythmias and SUDEP (Kerling, et al. 2009). Results presented in this dissertation also demonstrated a cardiac dysfunction that was mediated by catecholamine disturbance. Potentially, there are three mechanisms increasing cardiac arrhythmias and mortality risk following SE or repeated seizures: 1) a sufficient period of tachycardia or bradycardia to produce an ischemia reperfusion event similar to cardiac stunning, 2) seizure induced postganglionic altered cardiac autonomic innervations and 3) altered higher brain centers that influence and control the heart and ANS. While this dissertation demonstrated SE induced cardiac stunning after 90 min of SE, the minimum period of SymNS mediated cardiac effects needed to produce a cardiac dysfunction during SE or repeated seizures is not known and requires further investigation. Additionally, histological evaluation of cardiac SymNS innervations and higher brain centers, such as the medulla, pons, and

amygdala, following SE or repeated seizures has not been performed and would contribute further information on similarities or difference between the two disorders.

Future Directions

Role of Hypothalamic Pituitary Adrenal Axis

SE-induced brain damage and inflammation may affect critical areas in the brain associated with autonomic tone and regulation of the hormonal systems (Devinsky 2004, Kanter, et al. 1991, Kanter, et al. 1996). Indeed, alterations to these systems may increase cardiac associated risk factors. As an example, alterations in the HPA axis in depression are known to increase mortality (Kanner and Balabanov 2002). Additionally, it is known that activation of the HPA axis by stressful stimuli from a physiological or psychological disturbance is an essential component of the body's homeostatic system (De Kloet, et al. 1998, Juruena, et al. 2004, Thomson and Craighead 2008, Tsigos and Chrousos 2002). However, the effects of SE on the HPA axis are not established, despite the fact that chronic stress and brain damage following seizure cessation would most likely contribute. Although there are many potential alterations to explore such as alterations in glucocorticoid and mineralocorticoid receptor expression, brain derived neurotrophic factor expression, or SSRI treatments, in clinical research the DEX/CRH challenge test has repeatedly demonstrated alterations of the HPA axis and is a well

established phenomenon in human aging and chronically stressful conditions (Heuser 1998). The combined DEX/CRH challenge test was designed to detect the inhibitory feedback system and could be used to determine the responsiveness of the HPA axis inhibitory feedback mechanism in rats following SE (Hatzinger, et al. 1996).

Cardiac and CNS Protection

Treatment with a ß -1 receptor antagonist demonstrated a beneficial effect and exposes a mechanism by which SE induces cardiac deficits. However, potential consequences of preventing tachycardia during SE on CNS damage are still not clear. Nevertheless, results of these studies demonstrate that cardio-protective therapy with a ß -1 receptor antagonist, which blocks tachycardia during SE, does not increase risk of CNS neuronal death or damage as a result of altered or diminished perfusion pressure. Further study is needed to evaluate the effects of hypertension and tachycardia during seizure activity on CNS damage. Such studies could use various administrations of ß-antagonists and evaluate brain damage using Fluoro-Jade B imaging or MRI scans (Nairismagi, et al. 2006). Moreover, we used atenolol, which does not cross the blood brain barrier and therefore did not alter seizure severity; future studies that pursue a therapeutic strategy to protect SE-patients from catecholamine induced cardiac damage should use

ß-antagonists that due cross the blood brain barrier as they may provide a beneficial anti-seizure effect in addition to tachycardiac inhibition.

SymNS Innervations

Myocardial injury resulting from either ischemia or cardiomyopathy can cause changes in the SymNS innervations of cardiac tissue resulting in increased SymNS stimulation and nerve sprouting (Cao, et al. 2000b, Chen, et al. 2007, Igawa, et al. 2000, Li, et al. 2004). Changes in SymNS innervation are well established to increase the risk of ventricular hypertrophy, tachyarrhythmias, ventricular fibrillation, and sudden cardiac death (Airaksinen 1999, Anderson 2003, Cao, et al. 2000a, Cao, et al. 2000b, Chen, et al. 2007). However, the effects of SE on the SymNS innervations of the heart have not been systematically studied. It has been proposed that cardiac damage in SE is similar to that observed in the context of ischemia reperfusion injury (Harrigan, et al. 1994); thus it is anticipated that changes in the SymNS innervations of the heart following SE should be comparable. Therefore, future studies should histologically examine the neuronal innervations of cardiac tissue in SE-induced rats. This can be accomplished by comparison of sectioned rat hearts 2 weeks after SE that are stained for tyrosine hydroxylase, norepinephrine transporter, and the pan neuronal marker protein gene product in cardiac innervations (Li, et al. 2004). SE has been shown to affect autonomic function, predominantly producing SymNS

hyperactivity, in both the interictal and postictal periods. Changes in SymNS innervations of the heart combined with cardiac damage and remodeling may directly increase the susceptibility for a lethal arrhythmia.

Conclusion

Two mechanisms of catecholamine induced myocardial stunning have been proposed in the literature: (1) direct toxic effect on myocytes resulting in an excitation-contraction coupling dysfunction, indicative of a diastolic dysfunction; and (2) vasomotor constriction or spasm of the coronary microcirculation that results in a reversible ischemia/reperfusion injury (Bolli 1990). In either case, both mechanisms may lead to intracellular Ca^{2+} overload or oxidative stress resulting in catecholamine induced injury and may represent a form of myocardial stunning (Bolli 1990, Gao, et al. 1995). The pattern and the time course of LV dysfunction in these experiments demonstrate the most salient features of acute catecholamine induced myocardial stunning, including rapid development of profound LV diastolic dysfunction; significant hemodynamic compromise; a large discrepancy between the severity of LV contractile impairment and a modest rise in serum markers of myocardial damage (such as cTnI); complete reversal of myocardial dysfunction within weeks of SE cessation; and cardiac protection by inhibition of adrenergic activation of the heart. These results also suggest that additional treatment of LV diastolic dysfunction, caused by SE, may be

provided by relieving myocardial ischemia during seizures, improving systolic function with inotropic agents postseizure, or decreasing cardiac distention (Little and Applegate 1993).

The importance of using several animal models to study SE related mortality in this dissertation allowed for insights into a variety of mechanisms that contribute to increased mortality risk and to the initiation of sudden death. In this dissertations two models were used, Li-pilocarpine and SSLSE. While both models similar produced SE, the SSLSE model allowed for investigation of seizure induced cardiac effects without the muscarinic effect of pilocarpine. Regardless of the model used, similar cardiac dysfunctions were evident in both and were demonstrated by cardiac electrophysiology, direct LV pressure measurements and noninvasive MRI techniques. Together these consistent results strongly suggest that the cardiac effect produced by SE are model independent and are a direct result of seizure activity.

Overall, we conclude that SE produces sympathetically mediated cardiac dysfunction that mimics cardiac stunning in humans, which increases mortality and morbidity. Further, this cardiac dysfunction and the associate mortality risk can be reduced by pharmacotherapy during SE with a ß-receptor antagonist. Moreover, results presented in this dissertation provide a major mechanism by which prolonged seizures and subsequent sympathetic activation can cause neurogenically mediated cardiac stunning. Fortunately,

cardiac stunning is a potentially preventable and treatable disorder that warrants cardiac monitoring of all patients following severe and prolonged seizure activity. Future studies should further investigate potential mechanisms and treatment strategies to reduce mortality and morbidity in the period following SE.

References

Airaksinen KE. (1999) Autonomic mechanisms and sudden death after abrupt coronary occlusion. *Ann Med* 31:240-245.

Anderson KP. (2003) Sympathetic nervous system activity and ventricular tachyarrhythmias: recent advances. *Ann Noninvasive Electrocardiol* 8:75-89.

Benowitz NL, Simon RP, Copeland JR. (1986) Status epilepticus: divergence of sympathetic activity and cardiovascular response. *Ann Neurol* 19:197-199.

Bolli R. (1990) Mechanism of myocardial "stunning". *Circulation* 82:723-738.

Bolli R, Marban E. (1999) Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* 79:609-634.

Cao JM, Chen LS, KenKnight BH, Ohara T, Lee MH, Tsai J, Lai WW, Karagueuzian HS, Wolf PL, Fishbein MC, Chen PS. (2000a) Nerve sprouting and sudden cardiac death. *Circ Res* 86:816-821.

Cao JM, Fishbein MC, Han JB, Lai WW, Lai AC, Wu TJ, Czer L, Wolf PL, Denton TA, Shintaku IP, Chen PS, Chen LS. (2000b) Relationship between regional cardiac hyperinnervation and ventricular arrhythmia. *Circulation* 101:1960-1969.

Chen LS, Zhou S, Fishbein MC, Chen PS. (2007) New perspectives on the role of autonomic nervous system in the genesis of arrhythmias. *J Cardiovasc Electrophysiol* 18:123-127.

Choi YH, Cowan DB, Wahlers TC, Hetzer R, Del Nido PJ, Stamm C. (2010) Calcium sensitisation impairs diastolic relaxation in post-ischaemic myocardium: implications for the use of Ca(2+) sensitising inotropes after cardiac surgery. *Eur J Cardiothorac Surg* 37:376-383.

De Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. (1998) Brain corticosteroid receptor balance in health and disease. *Endocr Rev* 19:269-301.

Devinsky O. (2004) Effects of Seizures on Autonomic and Cardiovascular Function. *Epilepsy Curr* 4:43-46.

Gao WD, Atar D, Backx PH, Marban E. (1995) Relationship between intracellular calcium and contractile force in stunned myocardium. Direct evidence for decreased myofilament Ca2+ responsiveness and altered diastolic function in intact ventricular muscle. *Circ Res* 76:1036-1048.

Harrigan T, Bureau YR, Persinger MA, Parker GH. (1994) Prevention of sudden cardiac death by the atypical neuroleptic acepromazine following status epilepticus in rats. *Life Sci* 54:PL457-462.

Hatzinger M, Reul JM, Landgraf R, Holsboer F, Neumann I. (1996) Combined dexamethasone/CRH test in rats: hypothalamo-pituitary-adrenocortical system alterations in aging. *Neuroendocrinology* 64:349-356.

Heuser I. (1998) Anna-Monika-Prize paper. The hypothalamic-pituitary-adrenal system in depression. *Pharmacopsychiatry* 31:10-13.

Igawa A, Nozawa T, Yoshida N, Fujii N, Inoue M, Tazawa S, Asanoi H, Inoue H. (2000) Heterogeneous cardiac sympathetic innervation in heart failure after myocardial infarction of rats. *Am J Physiol Heart Circ Physiol* 278:H1134-1141.

Juruena MF, Cleare AJ, Pariante CM. (2004) [The hypothalamic pituitary adrenal axis, glucocorticoid receptor function and relevance to depression]. *Rev Bras Psiquiatr* 26:189-201.

Kanner AM, Balabanov A. (2002) Depression and epilepsy: how closely related are they? *Neurology* 58:S27-39.

Kanter RK, Erickson JT, Millhorn DE. (1991) Activation of the c-fos gene in prodynorphin- and proenkephalin-expressing cells of the nucleus tractus solitarius after seizures. *Exp. Neurol.* 129:290-298.

Kanter RK, Strauss JA, Sauro MD. (1996) Comparison of neurons in rat medulla oblongata with fos immunoreactivity evoked by seizures, chemoreceptor, or baroreceptor stimulation. *Neurosci.* 73:807-816.

Kerling F, Dutsch M, Linke R, Kuwert T, Stefan H, Hilz MJ. (2009) Relation between ictal asystole and cardiac sympathetic dysfunction shown by MIBG-SPECT. *Acta Neurol Scand* 120:123-129.

Li W, Knowlton D, Van Winkle DM, Habecker BA. (2004) Infarction alters both the distribution and noradrenergic properties of cardiac sympathetic neurons. *Am J Physiol Heart Circ Physiol* 286:H2229-2236.

Little WC, Applegate RJ. (1993) Congestive heart failure: systolic and diastolic function. *J Cardiothorac Vasc Anesth* 7:2-5.

Nairismagi J, Pitkanen A, Kettunen MI, Kauppinen RA, Kubova H. (2006) Status epilepticus in 12-day-old rats leads to temporal lobe neurodegeneration and volume reduction: a histologic and MRI study. *Epilepsia* 47:479-488.

Schuele SU. (2009) Effects of seizures on cardiac function. *J Clin Neurophysiol* 26:302-308.

Thomson F, Craighead M. (2008) Innovative Approaches for the Treatment of Depression: Targeting the HPA Axis. *Neurochem Res* 33:691-707.

Tsigos C, Chrousos GP. (2002) Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res* 53:865-871.

Walton NY. (1993) Systemic effects of generalized convulsive status epilepticus. *Epilepsia* 34 Suppl 1:S54-58.